ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 21 SEPTEMBER, 1937 NUMBER 5

THE FUNCTIONAL EFFICIENCY OF TRANSPLANTED ADRENAL CORTICAL TISSUE

LELAND C. WYMAN AND CAROLINE TUM SUDEN
From the Physiological Laboratory, Boston University School of Medicine and the Evans Memorial, Massachusetts Memorial Hospitals
BOSTON, MASSACHUSETTS

Ever since Jaffe and Plavska (1, 2) showed that the adrenal cortex may be transplanted successfully in rats and guinea pigs, the results of certain experiments have led to the tacit assumption that autoplastic cortical transplants in adrenalectomized rats fully replace the function of the excised glands. The results reported here together with those recently published by Ingle (3) show that this assumption is not correct.

Adrenalectomized rats having autoplastic cortical transplants in the abdominal muscles have been shown to be like normal rats with respect to general health, reproduction, resistance to diphtheria toxin (4), blood sugar (5), blood non-protein and urea nitrogen (6), blood volume (7), reaction to cold (8, 9), and growth rate (10). Adrenalectomized rats with intra-ovarial transplants have been reported to be normal with respect to withstanding anesthesia and the administration of distilled water, work performance (11), and activity level (12). It was found, however, that adrenalectomized rats from a mixed stock of colored varieties with intramuscular abdominal transplants were as susceptible to histamine poisoning and to anaphylactic shock as adrenalectomized rats (4, 13, 14). This led us to attribute this susceptibility entirely to loss of the adrenal medulla, especially since treatment with adrenalin somewhat alleviated this condition. Perla and Gottesman (15, 16), on the other hand, found that treatment of adrenalectomized rats with cortin decreased the susceptibility and therefore attributed it to loss of the cortical function. They suggested a histamine susceptibility test as a means of biological assay of cortical hormone. These two contradictory opinions have stood unexplained, and other authors have accepted now one and now the other opinion (17).

Ingle (3) found that adrenalectomized rats having intraovarial trans-

plants of cortical tissue, or rats with the adrenal medulla destroyed, showed a resistance to histamine much greater than that of the adrenalectomized rats but still inferior to that of normal animals. He was able to increase the resistance of rats lacking the adrenal medulla by treatment with epinephrine, but not by treatment with cortin. He concluded, therefore, that both parts of the glands are concerned in protecting the animals against histamine shock, and suggested that our previous findings might be explained according to his observations (unpublished) that although intramuscular transplants may maintain life and growth, they cannot maintain normal resistance of the animal to stress as do intra-ovarial transplants.

In the meantime we had continued with the problem stimulated by a similar suggestion, made to us by Professor F. A. Hartman, that perhaps transplanted tissue although capable of maintaining life is not able to function as fully as the intact gland in an emergency such as acute histamine poisoning.

METHODS AND RESULTS

An inbred strain of albino rats was used. Adrenal transplantation was made at 2 to 3 months of age by removing both glands, bisecting them, and placing the halves in the spinotrapezius muscle and beneath the transversus abdominis on each side. Histamine tests were made 2 months or more after this operation. In the adrenalectomized series the rats averaged 6 months old and were used 3 weeks after operation. Ergamine acid phosphate (Burroughs Wellcome and Co.) and eschatin (Parke, Davis and Co.)[1] were used. Carotid blood pressures were recorded by the mercury manometer, under urethane anesthesia.

Histamine Tolerance Tests. To test the susceptibility to histamine of rats with various degrees of cortical protection, ergamine acid phosphate in a 5 per cent aqueous solution was injected intraperitoneally. The dosage was on the basis of mg. per 100 gm. of body weight. The results are summarized in table 1.

The lethal intraperitoneal dose of histamine for normal rats has been shown to be more than 100 mg. per 100 gm., and that for adrenalectomized rats 10 mg. per 100 gm. or less (4). These facts were confirmed for the strain used in these experiments.

Although the lethal dose of histamine for rats with dorsal intramuscular transplants varied from 15 to 40 mg. per 100 gm., it appeared that the M.L.D. was 40 mg. or slightly more; that is, about one-half that for normal rats and 4 or 5 times that for adrenalectomized rats. This differs from our previous finding that transplants were as susceptible as rats with no cortical tissue (4) and agrees well with that of Ingle (3). The rats used in our previous experiments differed from those in this series in three major respects; site of transplantation, diet, and strain.

In our previous series we used abdominal intramuscular transplants. We prepared, therefore, a similar series from our present strain for comparison with the dorsal transplants. As shown in the table, their susceptibility to

[1] We are indebted to Parke, Davis and Co., for kindly supplying a portion of the eschatin used.

TABLE 1.

Description	No. of rats	Treatment Eschatin	Histamine mg./100 gm.	Survived	Died
Controls	11	none	50	11	0
Control	1	none	100	1	0
Adrenalectomized	7	none	10	0	7
Transplants					
Dorsal autoplastic	7	none	10	7	0
Abdominal autoplastic	10	none	10	10	0
Dorsal autoplastic	5	none	15	3	2
Dorsal autoplastic	5	none	20	3	2
Abdominal autoplastic	9	none	20	7	2
Dorsal autoplastic	7	none	30	1	6
Dorsal homoplastic	6	none	30	4	2
Dorsal autoplastic	8	none	40	1	7
Abdominal autoplastic	10	none	40	4	6
Dorsal homoplastic	5	none	40	3	2
Dorsal autoplastic	6	6 days	40	1	5
Dorsal autoplastic	5	1 day	30	3	2
Dorsal autoplastic	8	1 day	40	5	3
Dorsal homoplastic	6	1 day	40	5	1
Abdominal autoplastic	7	1 day	40	6	1
Summary of Treatment					
Transplants	36	none	30 & 40	13	23
Transplants	26	1 day	30 & 40	19	7

histamine was not less than that of the dorsal transplants. This indicates that the difference in the site of intramuscular transplantation cannot account for our previous results.

Since 1928 we had changed the diet of our colony from one consisting chiefly of bread, milk and mixed grains to one of Purina Dog Chow. According to the analysis of this product it is relatively higher in Na (0.67 per cent) and Cl (0.68 per cent) than the old diet. Although the potency of the secretion of the transplanted cortical tissue may have been enhanced by the additional salt, the relatively high percentage of K (0.56 per cent) should have been unfavorable. We have not yet tested the effect of the change in diet, but the similarity of the transplanted animals on the two diets in all other respects leads us to believe that the previous one was not especially deficient in factors necessary for adequate cortical function.

TABLE 2. MORTALITY RATE AFTER HISTAMINE IN RATS WITH VARIOUS DEGREES OF CORTICAL PROTECTION

Description	No. of rats	Histamine mg./100 gm.	Died <2 hrs.		Died <4 hrs.		Died <6 hrs.		Total	
			No.	%	No.	%	No.	%	No.	%
Adrenalectomized	7	10	5	71.5	6	85.8	7	100	7	100
Cortical transplants	36	30 and 40	7	18.9	14	37.8	18	48.6	23	63.5
Eschatin-treated cortical transplants	32	30 and 40	1	3.1	7	21.7	10	31.0	12	37.2

It is left for us to conclude that the difference in strain, mixed colored and hooded varieties vs. inbred pure albino, was a major factor responsible in our earlier work for the relative inefficiency of the transplanted tissue in histamine shock. It has been shown that rats of different colonies differ in their susceptibility to adrenal insufficiency (18, 19).

Although the majority of the transplanted rats used had autoplastic grafts of cortical tissue, 10 with homoplastic grafts were tested. The protection against histamine afforded by such tissue was fully equal to that afforded by autoplastic grafts and is further evidence that homoplastic transplants, when successful, are functional (20).

That transplanted tissue was responsible for a certain amount of resistance to histamine was further demonstrated in 2 rats which had survived the administration of 40 mg. per 100 gm. and were killed by 10 mg. after removal of their grafts.

To see if additional protection would be afforded by the administration of a cortical extract, 0.5 cc. of eschatin was injected intraperitoneally twice daily for 6 days, the last dose being given 2 hours before a test dose of histamine. In 4 other series 3 one cc. doses of eschatin were given at 12-hour intervals, the last one 2 hours prior to the histamine test, and 1 cc. 2 hours after the histamine. From the data presented in tables 1 and 2 and in figure 1 it can be seen that this latter therapy increased the percentage of survivors and extended the survival period of the fatal cases. The benefit, however, was moderate and was in proportion to the amount of eschatin given just prior to and during the histamine shock rather than to the extent of the period of therapy. This harmonizes with the suggestion mentioned above, namely that the efficiency of cortical tissue in combating acute histamine poisoning depends on the amount of hormone which the tissue is able to mobilize during stress. The efficiency of the preparation used was undoubtedly not equal to that of physiologically produced hormone. Gottesman and Perla (16) were not able to raise the resistance of adrenalectomized rats entirely to the normal level by repeated injections of large amounts of cortin. To restore normal resistance would probably require epinephrine in amounts comparable to that produced by the adrenal medulla during histamine shock plus a certain additional amount of effective cortical influence. The latter factor would vary with the cortical preparation used and may account for different results in the experiments of different investigators.

Blood Pressure Experiments. We had previously shown that the minimal dose of histamine necessary to produce a permanent fall in blood pressure was from 0.3 to 0.5 mg. per 100 gm. body weight for normal rats, and from 0.05 to 0.1 mg. for adrenalectomized rats of the colored strains, with or without transplanted cortical tissue (14). In 18 blood-pressure experiments on rats of our present white strain we confirmed the above critical depressor dose for normal rats, but found that at least 0.2 mg. per 100 gm. was required to produce a permanent fall in the majority of the transplanted rats (6 of 8). This, being between the values for the two types of animal in the previous work, is consistent with the results of the histamine tolerance tests. Intraperitoneal

injection of two 1 cc. doses of eschatin, 3 and 1 to 2 hours before the experiment, did not alter the vascular responses to histamine. Any additional protection against histamine shock afforded by injected cortical extract is apparently not reflected in the immediate vascular response. Eschatin did, however, improve the initial blood pressure. The range of initial blood pressure was 114 to 133 mm. Hg for normals (3 rats), 114 to 134 for transplants (4), 138 to 150 for eschatin-treated transplants (7), and 114 to 132 for saline injected control transplants (4).

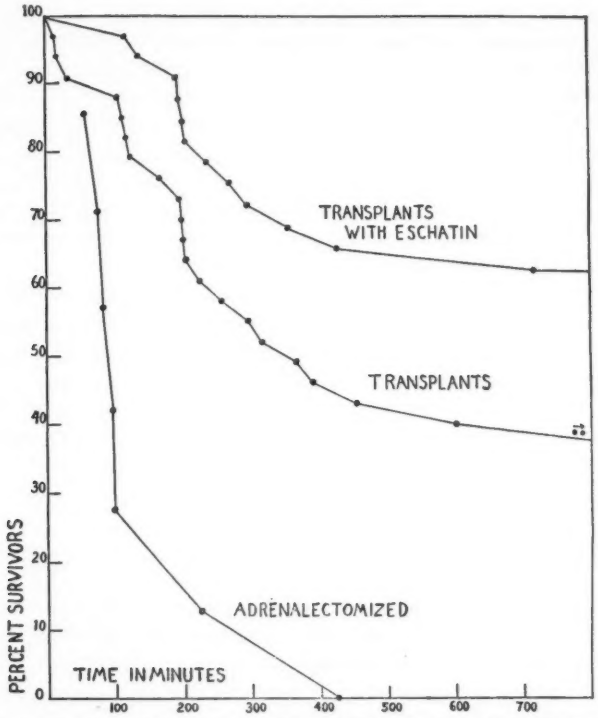


Fig. 1. Survival time of eschatin-treated and untreated rats with cortical transplants after 30 or 40 mg. of histamine per 100 gm., and adrenalectomized rats after 10 mg. per 100 gm. Histamine injected at 0 min.

DISCUSSION

We agree with Ingle (3) that the increased susceptibility of the rat to histamine after adrenalectomy depends both on medullary and on cortical lack. Transplanted cortical tissue can supply a variable amount of protection against histamine, its efficiency varying with the individual and with the colony. Additional protection may be afforded by administering cortical extracts just before or during histamine poisoning; but a certain degree of cortical efficiency is still lacking. To restore normal resistance would require medullary function as well, or comparable amounts of adrenalin.

Ingle, Hales and Haslerud have said that the site of transplantation is

important with respect to the efficiency of transplanted tissue; that transplants on the spleen, in mesentery, in muscle or under the skin may maintain life and normal growth, but may not maintain resistance to stress; whereas those in the ovary are more efficient during stress (11, 3). They attribute this to the vascularity of the ovary, and state that sections of transplants in other sites show large amounts of necrotic tissue and a meager blood supply (11). Although we have not investigated the efficiency of intra-ovarial transplants, we are not yet inclined to agree that intramuscular transplants are distinctly and consistently less efficient than those in other sites. The susceptibility to histamine of rats with dorsal or abdominal intramuscular transplants, reported here, agrees well with Ingle's findings in rats with intra-ovarial transplants (3). It has frequently been our experience that adrenalectomized rats possessing internal masses of cortical tissue, either accessories or regenerated from operative rests, have behaved the same as those with intramuscular transplants in histamine tolerance tests. All of our long established transplants, irrespective of site, have shown no necrotic tissue and have been well vascularized, even reacting with a marked hyperemia in acute fatal conditions. We believe, therefore, that site of transplantation is a minor factor in the efficiency of transplants which have become established.

Goldzieher and Barishaw have shown that transplantation of cortical tissue may be of value in certain cases of Addison's disease (21). They suggest that the eventual death of their patient, who possessed surviving cortical transplants morphologically in good condition, may have occurred because the grafts, while able to supply an ordinary amount of hormone for 6 months, were not able to meet a sudden demand for more in an emergency. This quantitative viewpoint is consistent with our conclusions.

SUMMARY

The lethal intraperitoneal dose of histamine for adrenalectomized rats of an inbred white strain having intramuscular transplants of adrenal cortical tissue was variable but averaged about one-half that for normal rats, and 4 or 5 times that for rats having no cortical tissue. Homoplastic transplants afforded protection against histamine equal to that given by autoplastic grafts. Administration of a cortical extract (eschatin) increased the percentage of survivors and extended the survival period of the fatal cases, but did not restore normal resistance. The results of blood-pressure experiments were consistent with those of the histamine tolerance tests.

It is concluded that increased susceptibility of the rat to histamine after adrenalectomy depends both on medullary and on cortical lack. Transplanted adrenal cortical tissue, or that regenerated from accessories or operative rests, although capable of maintaining life, general health and resistance to some forms of stress, is not able to function as efficiently as does the normal gland in acute emergencies such as histamine poisoning. The efficiency of transplanted tissue in this respect varies both individually and with colony, and probably depends upon the amount of hormone which can be quickly mobilized.

REFERENCES

1. Jaffe, H. L., and A. Plavska: Proc. Soc. Exper. Biol. & Med. **28:** 528. 1926.
2. Jaffe, H. L.: Am. J. Path. **2:** 421. 1926.
3. Ingle, D. J.: Am. J. Physiol. **118:** 57. 1937.
4. Wyman, L. C.: Am. J. Physiol. **87:** 29. 1928.
5. Wyman, L. C., and B. S. Walker: Am. J. Physiol. **89:** 215. 1929.
6. Wyman, L. C., and B. S. Walker: Am. J. Physiol. **89:** 349. 1929.
7. Wyman, L. C., and C. tum Suden: Am. J. Physiol. **94:** 579. 1930.
8. Wyman, L. C., and C tum Suden: Am. J. Physiol. **89:** 362. 1929.
9. Wyman, L. C., and C. tum Suden: Proc. Soc. Exper. Biol. & Med. **30:** 61. 1932.
10. Wyman, L. C., and C. tum Suden: Am. J. Physiol. **101:** 662. 1932.
11. Ingle, D. J., W. M. Hales and G. M. Haslerud: Am. J. Physiol. **114:** 653. 1936.
12. Ingle, D. J., and R. E. Harris: Am. J. Physiol. **114:** 657. 1936.
13. Wyman, L. C.: Am. J. Physiol. **89:** 356. 1929.
14. Wyman, L. C., and C. tum Suden: Am. J. Physiol. **99:** 285. 1932.
15. Perla, D., and J. Marmorston-Gottesman: Proc. Soc. Exper. Biol. & Med. **28:** 650. 1931.
16. Marmorston-Gottesman, J., and D. Perla: Proc. Soc. Exper. Biol. & Med. **28:** 1022. 1931.
17. Schultzer, P.: J. Physiol. **87:** 222. 1936.
18. Gaunt, R.: Am. J. Physiol. **103:** 494. 1933.
19. Gaunt, R., J. H. Gaunt, and C. E. Tobin: Proc. Soc. Exper. Biol. & Med. **32:** 888. 1935.
20. Wyman, L. C., and C. tum Suden: Endocrinology, **21:** 523. 1937.
21. Goldzieher, M. A., and S. B. Barishaw: Endocrinology **21:** 394. 1937.

THE USE OF ESTRIN IN THE TREATMENT OF ECLAMPSIA

EVAN SHUTE
From the Department of Obstetrics and Gynaecology, University of Western Ontario
LONDON, CANADA

A multitude of theories as to the origin and treatment of eclampsia have been suggested. Recently Smith (1) demonstrated that both the blood and placentae of eclamptic women were characterized by a low estrin content and an excess of the placental or pregnancy-urine gonadotropic hormone. Heim (2), however, observed a very high excretion of both estrin and gonadotropic hormone in the urine of eclamptics. Bickenbach and Fromine (3) could find no increase in the blood content of estrin in 4 cases of eclampsia.

In the past three years 8 convulsive eclamptics have come under the observation of the author. Only 1 of the 8 displayed an excess of estrogenic substance in the blood. In another case, the only one in which a Friedman test was made, the prolan output in the urine was found to be very high.

Mild toxemias of late pregnancy of the abruptio placentae type have been treated with wheat germ oil with striking success (4). Apparently the oil neutralises the estrogenic substance which is found in excess in the blood of these cases (5). If eclamptic toxemias were to be treated analogously, an effort to control the excessive formation and excretion of prolan should be made. Reynolds (6) found that prolan and estrin were antagonistic in their effect on the uterine musculature. Jeffcoate (7) suggested that the presence of excess of estrin in some pregnant women prevented the accumulation of sufficient prolan in the urine to give positive pregnancy tests. The author had a patient 6-weeks pregnant whose blood showed an excess of estrogenic substance and whose urine would not give a positive Friedman test. A single dose of 8 drams of bulk ether-extracted wheat germ oil was administered in order to neutralise the estrin in the blood. In 24 hours the blood still showed some estrin excess but the Friedman test was a type II response. Another single dose of 6 drams of oil was given and in 24 hours the blood revealed no estrin excess while the urine gave a maximally positive Friedman test (only 48 hours after the first negative Friedman report had been received). A number of workers, notably Hawkinson (8), have helped cases of hyperemesis gravidarum, known to be characterized very often by an excessive excretion of prolan, by means of estrin therapy. The author has had a similar experience with hyperemesis.

These case reports were presented at the Kingston meeting of the Canadian Physiological Society, Oct. 30, 1936.

Accordingly we present the case history of a late pre-eclamptic and a severe convulsive eclamptic, both treated with estrin.

Mrs. J.T., aged 23 years, gravida one. Her last menses began Nov. 7, 1935. Her early pregnancy was undisturbed by nausea and she felt well throughout the whole gestation. On July 31, 1936, the author first saw her in consultation, when she was about 2 weeks short of term. At that time her blood pressure was 154/94, there was a slight oedema of the fingers and face, and no albuminuria. One week previously her doctor had noticed albuminuria and had 'restricted salt' in her diet and put her to bed. The child was active and the head floating. It appeared to be about the expected size and lay as a right occiput transverse. There had been no history of uterine hemorrhage, sacral backache nor uterine tenderness. No uterine tenderness was detectable at the time she was seen. A blood test revealed no excess of estrogenic substance, and accordingly we suspected that she was a true pre-eclamptic. She was put at absolute bed rest at home and a more rigid salt restriction was enforced.

On Aug. 4, 1936, her blood pressure was still the same, but in spite of the precautions taken, a marked albuminuria had developed. Accordingly she was sent into hospital for induction. A Friedman test, made on a catheterized morning urine sample, was very strongly positive with 1 cc. but negative with 0.25 cc. of the same sample. Similarly, the Visscher-Bowman test made on this urine sample was strongly positive. Before these reports had been received, a medical induction had been instituted on the morning of Aug. 5. No effect was observed. The author ruptured her membranes at noon when the blood pressure was 164/108. In the afternoon the patient developed spots before the eyes and the occipital headaches which had been present since early morning now rendered her so dizzy that she could not sit up in a chair. There were no labor pains that day. At 5:00 P.M. the blood pressure had risen to 174/106. She was then given 10,000 R.U. of estroform hypodermically and given orally 4 drams of emmenin every 3 hours. There were no pains during the night. At 9:00 P.M., however, the pressure had fallen to 162/96.

At 9:00 A.M. on Aug. 6, the blood pressure was the same. Her blood serum gave a test that was indefinite but rather suggested the presence of estrogenic substance. The Visscher-Bowman test was negative. She was given another 10,000 R.U. of estroform hypodermically. At 9:30 A.M. weak uterine contractions developed and soon became severe. There was some nausea and accordingly the emmenin had to be discontinued. By noon the cervix was dilated 7 cm. and was very soft. The pains were prolonged and very effective. She was given 6 grains of sodium amytal by mouth, developed slight disorientation and later proved to have had a good amnesia. At 3:00 P.M. there was deep transverse arrest of the head and an easy forceps delivery of a normal 7 lb. male was effected. The placenta came away readily and seemed unusually normal. Later in the day her blood pressure had fallen to 154/72. A blood sample taken at the time of her delivery was high in estrogenic substance, more than 2.5 R.U. per cc. of blood serum.

A catheter urine sample taken on the following morning was again strongly positive by the Visscher-Bowman test. By the 5th day post-partum her blood pressure had reverted to normal level. There was an uneventful course but for a transient lochiometra.

Mrs. J.W., aged 38 years, para II, gravida III. Her last menses began Nov. 21, 1935. Her first child was supposedly full term, but weighed only 4 lb. 3 oz. The next child was a difficult delivery at term and weighed 8 lb. 3 oz. As in her 2

former pregnancies, the first month of this pregnancy was characterized by severe nausea and vomiting. There was less than 20 lb. gain of weight during the whole pregnancy. Until the middle of July, 1936, her systolic pressure was no higher than 84 and her urinalyses were normal. Then the pressure began to rise and was 140 systolic in the 2 weeks before her entry into hospital. In that 2 weeks her breasts had 'suddenly filled up.'

Her previous history was of migraine headaches for years, peptic ulcer for years, and vitiligo.

On the evening of Aug. 13, she ate some green apples and during the night vomited. Next day there was diarrhea and severe epigastric distress unlike the old peptic ulcer pain. There was no headache and no visual disturbance. The epigastric pain was continuous and unaffected by posture or heat. At 10:30 P.M. on the night of the 14th, this epigastric pain disappeared. At 11:00 P.M. a severe headache over the vault was complained of, just as was noted with her menstrual periods. At midnight she was 'fussy.' At 2:00 A.M. she awakened with a violent headache which made her 'frantic,' but there was no epigastric distress. Her condition was unchanged at 4:00 A.M. At 7:00 A.M. she 'felt faint' and promptly had a convulsion. A colleague, Dr. Wilcox, was called, found her blood pressure 154/106, recognized the eclampsia, gave ¼ grain of morphia hypodermically, sent her into Victoria Hospital, and notified me. Dr. Wilcox observed that the reflexes were greatly exaggerated in the right leg and absent in the left. Her second convulsion occurred at 8:25 A.M. just as she reached the hospital. This was the first occasion on which either of us had seen this patient, as she had been attended up to this time by another physician who had just left the city for a vacation.

By the author's direction, the patient was given 20,000 R.U. of progynon-B intramuscularly. At 8:30 A.M. a retention catheter was inserted and the first portion of urine obtained sent to the laboratory for a Friedman test and a Visscher-Bowman prolan test. The Visscher-Bowman test was maximally positive and the Friedman test gave 'reaction II' in one follicle of one ovary, using ¼ cc. of urine. Blood was taken for the author's test for estrogenic substance, which proved to be quite negative. At 8:45 A.M. there was a third severe convulsion. At 9:00 A.M. an intravenous of 500 cc. of 20 per cent glucose was begun and as a fourth convulsion occurred during its administration, 2 cc. of 50 per cent magnesium sulphate was injected into the rubber tubing of the intravenous apparatus. The patient was in deep coma all this time. A very mild and final convulsion occurred at 10:30 A.M. At this time her blood pressure was 130/110, and she was tossing about in bed very restlessly. At 11:20 A.M. she was given 10 cc. of 20 per cent magnesium sulphate intramuscularly. At 12:20 P.M. she was given another 20,000 R.U. of progynon-B. At 1:05 P.M. 500 cc. of 20 per cent glucose solution was given by vein. She had by that time become quieter and the urine output since the catheter had first been inserted that morning had reached 4 ounces. At 2:45 P.M. another 10 cc. of 20 per cent magnesium sulphate was given hypodermically and this was the last dose of sedative administered hypodermically. The patient was nauseated at this time. At 4:00 P.M. her blood pressure had risen to 198/120. Four drams of emmenin given orally was vomited. At 6:00 P.M. the author ruptured the membranes through a cervix dilated to 4 cm., as the pressure seemed to be rising rapidly. At this time the patient was restless but controllable by loud and emphatic commands. She was dazed but the deep coma had lifted. Her blood pressure just afterward fell to 145/98. In the 5 hours previous to this she had excreted 2 more ounces of urine, which still gave a maximally positive Visscher-Bowman test. She took 2 drams of emmenin

by mouth every 4 hours. At 7:30 P.M. a blood sample showed some excess of estrogenic substance, and weak uterine contractions had just begun, coming fairly regularly. At 8:00 P.M. the blood pressure was 160/110. By 10:00 P.M. 3 more ounces of urine had been excreted, still very strongly positive for prolan by the V-B test. The weak uterine contractions continued during the night but by 3:00 A.M. no increase in cervical dilatation could be discerned. She was still 4 cm. dilated. She had gradually become less restless and by this time was fairly co-operative in a sluggish and dazed way. At 3:30 A.M. there was a sudden gush of 100 cc. of bright red blood, the pains at once became very strong, and she delivered spontaneously a 5 lb. 4 oz. living male child at 4:15 A.M. The placenta came away readily, revealing numerous large white infarcts extending through from one side of the placenta to the other. On the maternal aspect there were many large areas of yellow-brown, recently organized clots which had been leached out fairly well. There were no recent clots discernible. A blood test taken just after delivery revealed a weakly positive test for estrogenic substance.

At 8:30 A.M. on Aug. 16 her blood pressure had fallen to 124/88, from which it soon rose to 172/90 on the 19th and fell gradually to 148/86 on the 29th. The kidneys never closed down but secreted steadily. The retention catheter was removed at noon on Aug. 17. She remained rather vague and drowsy for 3 days. She had acute liver tenderness for that 3 days and some evidence of it for another 3 days.

She was given wheat germ oil to promote lactation, but this failed. A lochiometra developed with the fundus held near naval level in spite of doses of ergoklonin. Accordingly the oil was stopped, and she was given regular small doses of progynon-B hypodermically and emmenin orally to restore the uterine irritability. The fundus remained at about the same level, but after 2 days of such preparation, pituitrin acted energetically to expel the lochia and quickly lowered the fundus level to normal. An old pyelitis, which had given symptoms for the last 2 months of gestation, now flared up and it was difficult to put an old peptic ulcer case on the salt restriction required by her renal damage. She developed hot flashes on the estrin therapy and these were severe for several days, even lasting 4 days after the estrin had been discontinued. She was discharged Sept. 4, 1936, with same blood pressure, viz. 148/88, a slight residual right-sided pyelitis, and put on a salt and milk restriction in spite of her old ulcer story. Her blood had spontaneously become positive for estrogenic substance by this time, and she was quite unable to nurse her child.

These two detailed histories illustrate our present views. The first history is strongly suggestive of impending convulsions, although one can never say with certainty that such a patient must have had convulsions if left alone. In each case the hormonal situation was the same, viz., a high excretion of prolan and a low or normal blood content of estrogenic substance. In each case the administration of estrogenic substance very soon showed itself in both the blood serum and the onset of labor. Under the influence of estrin and sedative therapy the patients did not grow worse and the convulsive patient, indeed, rapidly improved objectively in spite of some rise of blood pressure. (This was the only reading made by an interne.) Convulsions ceased in the second case as estrin control was established, and this can scarcely be ascribed to the sedative therapy, as delivery occurred 13½ hours after the last dose of sedative and long after her drowsiness had largely disappeared. Estrin control was

established more readily than would be suspected, if the change in blood reaction and the onset of labor pains are any indication of that. Allowing as much time for the second dose of progynon to take effect as was taken by the first dose brings us to the time of the sudden hemorrhage and the development of unusually severe pains, a picture strongly suggestive of abruptio placentae, and if so, confirming our theory of the relationship of excess of estrogenic substance in the blood to the onset of abruptio placentae. This certainly suggests that estrin therapy, during pregnancy at least, should be used very cautiously and in small dosage. Eclamptic patients are difficult to induce, if not already in labor when the convulsions develop, and are notoriously liable to infection. The obstetrician has, therefore, often resorted to surgical means of delivery such as the hydrostatic bag or even Caesarian section, and a high morbidity has ensued, to say the least. The estrin type of therapy we have used may suggest an alternative and safer method of handling these cases.

The post-partum management is also of interest, perhaps. This is one of the cases in which wheat germ oil did not effect adequate lactation where it was needed. However, the uterus ceased to involute in spite of doses of ergoklonin, and we ascribed this subinvolution to a neutralisation of the estrin normally responsible in part, at least, for its irritability and contractility. We have indicated that we believe wheat germ oil and estrin are antagonistic in their actions. Accordingly the oil was stopped and estrogenic substances administered. Pituitrin, known to be synergistic to estrin, then acted very promptly and completely to lower the fundus level.

The author desires to thank British Drug Houses for supplies of estroform, the Schering Company for supplies of progynon-B, and Ayerst, McKenna and Harrison for supplies of emmenin.

Addendum: Since this report was sent to press the author has treated a postpartum eclamptic with estrin. She was delivered at 4:00 A.M. on June 17, 1937. At 8:00 A.M. on that day she had her first convulsion with a blood pressure of 124/86 and a maximal albuminuria. She had 3 more convulsions before 3:00 P.M. Although her blood serum revealed the presence of estrin, as though nature were endeavoring to neutralise her prolan, she was given 10,000 I.U. of progynon-B intramuscularly at 11:30 A.M. After 5:00 P.M. she rapidly recovered from her stupor. Indeed she seemed grossly normal by 7:00 P.M. when as much more estrin was given. A retention catheter demonstrated a steady and approximately normal excretion of urine throughout. The blood pressure did not change. By 4:00 A.M. the albuminuria was much decreased; by 6:00 A.M. there was none, and so she remained. Her postpartum course was normal after this first eventful day.

REFERENCES

1. Smith, G. V. S., and O. W. Smith: Surg., Gynec. & Obst. **61:** 175. 1935.
2. Heim, K. Klin. Wchnschr. **13:** 1614. 1934.
3. Bickenbach, W., and H. Fromme. Klin. Wchnschr. **14:** 496. 1935.
4. Shute, E. V.: J. Obst. & Gynaec. Brit. Emp. **44:** 121. 1937. Am. J. Obst. & Gynec. **33:** 429. 1937.
5. Shute, E. V.: J. Obst. & Gynaec. Brit. Emp. **43:** 74. 1936. J. Obst. & Gynaec. Brit. Emp. **44:** 253.
6. Reynolds, S. R. M.: Am. J. Obst. & Gynec. **29:** 630. 1935.
7. Jeffcoate, T. N. A.: J. Obst. & Gynaec. Brit. Emp. **39:** 67. 1932.
8. Hawkinson, L. F.: Minnesota Med. **19:** 519. 1936.

COMPLETE AND INCOMPLETE ESTROGENIC HORMONES, ARISING FROM DIFFERENT SITES IN THE RAT'S OVARY

S. C. FREED AND SAMUEL SOSKIN
From the Department of Metabolism and Endocrinology,[1]
Michael Reese Hospital
CHICAGO, ILLINOIS

Two main views regarding the site of origin of estrogenic hormone in the ovary have been advanced. Zondek (1) believes that it arises from the theca, while Allen et al. (2) hold the granulosa cells responsible. We wish to present evidence that the rat's ovary secretes at least two estrogenic hormones, one from the theca and the other from the granulosa. These two estrins can be readily distinguished by their effects on the accessory sex organs of the animal.

There is now considerable evidence to indicate that the estrus phenomenon is not a simple process resulting from the action of a single hormone. We have recently shown that there are at least three phases of the estrus cycle which cannot be reproduced by the administration of the estrogenic hormone of the ovarian follicle to castrate rats (3). That the follicular hormone is not the only estrogenic substance involved is also indicated by the recent isolation of a new estrin from hog's ovaries, (4) and the preparation of an extract of interstitial tissue with unusual estrogenic properties (5).

Our method of approach to this problem depended on previous work in which we compared the response of the ovaries and the uterus in the immature guinea pig and rat respectively, to the administration of human pregnancy urine (6). This work gave some indication that the response of the uterus in the two animals, and in different stages of immaturity in each animal, depended upon the extent to which the APL substance had influenced the theca or granulosa or both at any given time. In the present work we have made a detailed correlation of the 4 stages of ovarian response to APL in the immature rat, which we have previously described, with the coincident changes in the uterus and vagina.

METHODS AND RESULTS

Infantile female rats, varying in age from 6 to 21 days, were given daily injections of 25 R.U. of APL for 4 days. On the fifth day the rats were sacrificed. Table 1 summarizes the results of gross and histologic examination of the ovaries, uterus and vagina in these animals.

It may be seen that during Stage II, the ovarian response to APL was confined to the luteinization of the theca and interstitial cells. No change could be demonstrated in the granulosa cells, and there was no increase in the

[1] Aided by a Grant from the Committee on Scientific Research of the American Medical Association.

size of the follicles. Nevertheless the ovary, at this stage, was capable of inducing estrus as judged by vaginal cornification and uterine enlargement. But since the endometrium had not changed from its infantile cuboidal state, it is apparent that this estrus lacked an essential feature of true or complete estrus. The estrogenic hormone arising from theca and interstitial cells must therefore be characterized as 'incomplete'.

Endometrial proliferation appeared in Stage IV. Its advent coincided closely with the first appearance of enlarged ovarian follicles. It is apparent that

THE RESPONSE OF THE UTERUS AND VAGINA TO OVARIAN STIMULATION BY APL, AT DIFFERENT STAGES OF IMMATURITY IN THE RAT

Stage	Number of Rats	Vagina	Uterine[1] Weight mg.	Endometrium	Ovary
I 0–6	4	Infantile	2–4 (2–4)	Cuboidal (infantile)	Primary follicles
II *a*) Early 8–12 Days	14	Cornification	22 (8)	Same as Stage I	Luteinization of theca and interstitial cells. No change in the granulosa.
b) Late 14–15 Days	8	Cornification and canalization	66 (12)	"	"
III 16–17 Days	4	Same as Stage II b	74 (14)	Same as Stage I	Same as Stage II but granulosa in transitional state of luteinization.
IV 17 Days	6	Same as Stage II (b)	92 (16)	Low columnar	Onset of follicular growth.
18–19 Days	6	"	98 (18)	Moderate to high columnar	Follicular growth with corpora lutea formation.
20 Days	10	"	110 (22)	High columnar	Same as above but more advanced.

[1] The bracketted figures indicate approximate uterine weight at a corresponding period in untreated control animals.

complete estrus occurred only after the granulosa had responded to APL stimulation, and the estrogenic hormone from this source may be characterized as 'complete'.

There was the possibility that the incomplete estrus reaction of the uterus, which we observed in Stages II and III, might have been due to a refractory state of the uterus rather than the character of the estrin elaborated by the ovary. This possibility was excluded by the fact that 14-day-old rats injected with pure theelin developed full-blown endometrial proliferation. The uterine changes which we observed with APL may therefore be taken as a true reflection of ovarian function.

DISCUSSION

That the rat's ovary elaborates two estrins, one complete and the other incomplete, is not difficult to reconcile with our present knowledge of the estrogenic substances. Dodds (7) has demonstrated the possibility of obtaining substances which give rise to various stages or degrees of estrus, by chemical manipulation of the estrogenic compounds. Korenchevsky (8) has listed various sterols, related to estrin, as 'pseudo-estrogenic' because of their partial effects on the female accessory organs. Even the term 'complete estrin' can be used only in a relative sense. Trihydroxyestrin, for example, has only a slight effect on the uterus of the infantile rat, as compared to its action on the vagina. Also, as we have previously reported (9), dihydroxyestrin is more effective than ketohydroxyestrin in suppressing pituitary gonadotropic activity, when each is administered in amounts which have an equal effect as regards vaginal cornification. Thus the estrin resulting from stimulation of the theca may be cited as a naturally occurring incomplete estrin, analogous to many of the products of the biochemical laboratory.

The consideration of the estrus phenomenon by its component parts, instead of as a unit, may lead to an understanding of experimental results which, hitherto, have been difficult to explain. Thus, we have previously reported (10) that rats treated with the urine from a case of teratoma testis reacted in a manner similar to, but not identical with, those treated with pregnancy urine. Luteinization of the follicles occurred, but the corpora differed from those found in the Aschheim-Zondek reaction in that they were avascular, the lutein cells touching each other on all sides. The uterus was enlarged, pale and not distended, while the endometrium remained cuboidal, as in Stage II of our present results. In the light of our present work we may surmise that this incomplete estrus resulted from theca cell stimulation, and that the avascular granulosa did not secrete sufficient complete estrin to induce endometrial proliferation.

Another instance in which an incomplete estrus was obtained is of considerable interest (11). This occurred in infantile rats following the administration of neutral extracts of the anterior hypophysis, combined with blood serum for augmentation purposes. In this case the corpora lutea which resulted were abundantly supplied with capillaries. However, examination of the ovaries 48 hours after the first administration of the extract, revealed the fact that the granulosa of most follicles was far advanced in the process of luteinization, much beyond that seen in ovaries subjected to APL for a similar length of time. It may be that the lack of endometrial proliferation in these experiments was due to the rapidity with which the granulosa was luteinized, no opportunity being given for the elaboration of its estrogenic hormone. If this be so, a greater regard for the time element in the interpretation of such work is indicated.

Other workers have reported similar variations in the estrus phenomenon. Fluhmann (12) has noted that APL and anterior pituitary extract elicit different uterine responses in infantile rats. Katzman and Doisy (13) have observed that gonadotropic extracts of urine may produce peculiar responses such

as uterine estrus without ovarian enlargement and vice versa. Owen and Prince (14) have listed several ovarian-uterine response combinations following the administration of teratoma testis urine extracts. We shall not attempt to explain all these results, but it seems logical to conclude that a reconsideration of the experimental data in the light of the present work, would aid in their interpretation. Such a study should include not only the distinction between thecal and granulosal activity, but also the speed of the cell changes which are induced.

Finally it is suggested that, if it can be shown that the present results in the rat also apply to the human, the interpretation of the clinical manifestations resulting from ovarian dysfunctions may be approached from a new basis.

SUMMARY

The ovary of the rat secretes at least two estrogenic hormones, one from the theca and the other from the granulosa. The estrin of the theca is incomplete. It does not induce endometrial proliferation. The follicular estrin is complete, in a relative sense.

The results herein reported aid in the interpretation of previous, hitherto unexplained, experimental work of the authors and others, and the possibility of applying these results to the interpretation of clinical disorders in the human is suggested.

REFERENCES

1. Zondek, B.: Hormone des Ovariums und des Hypophysenvorderlappens, Julius Springer, Vienna, 1935.
2. Allen, E., E. A. Doisy, B. F. Francis, H. V. Gibson, L. L. Robertson, C. E. Colgate, W. B. Countz and C. G. Johnston: Am. J. Anat. **34:** 133. 1924.
3. Freed, S. C., and S. Soskin: Proc. Soc. Exper. Biol. & Med. **35:** 409. 1936.
4. Andrew, R. H., and F. Fenger: Endocrinology **20:** 563. 1936.
5. Marlow, H. W.: Endocrinology **20:** 239. 1936.
6. Freed, S. C., and A. Coppock: Endocrinology **20:** 81. 1936.
7. Dodds, E. C.: Harvey Lectures, 1935.
8. Korenchevsky, V.: Biochem. J. **31:** 475. 1937.
9. Freed, S. C., and S. Soskin: Endocrinology **20:** 863. 1936.
10. Freed, S. C., and A. Coppock: Proc. Soc. Exper. Biol. & Med. **32:** 589. 1935.
11. Freed, S. C.: Proc. Soc. Exper. Biol. & Med. **33:** 309. 1935.
12. Fluhmann, C. F.: Endocrinology **17:** 550. 1933.
13. Katzman, P. A., and E. A. Doisy: J. Biol. Chem. **106:** 125. 1934.
14. Owen, S. E., and L. H. Prince: J. Lab. & Clin. Med. **22:** 431. 1937.

COMPARATIVE ACTION OF TESTOSTERONE COMPOUNDS, OF ESTRONE AND OF COMBINATIONS OF TESTOSTERONE COMPOUNDS AND ESTRONE ON THE ANTERIOR HYPOPHYSIS[1]

J. M. WOLFE AND J. B. HAMILTON
From the Departments of Anatomy, Albany Medical College, Union University
ALBANY, NEW YORK
and Vanderbilt University Medical School
NASHVILLE, TENNESSEE

It has been reported that injection of large amounts of APL (anterior pituitary-like substance), simultaneously with estradiol (the benzoic acid ester) into normal male and female rats resulted in a modified or a partially suppressed reaction of the anterior hypophysis to estradiol (1). Thus in rats which received combined injections of APL and estradiol the degree of increase in the pituitary weight, and the degree of degranulation of the chromophilic cells, together with a reduction in their percentages and an increase in those of the chromophobes, were definitely less than in those rats which received estradiol alone. Such a suppression of the action of estradiol on the anterior lobe by the simultaneous injection of APL was not obtained in castrated rats. The present data indicate that injection of certain testosterone compounds[2] simultaneously with estrone,[3] also results in a modified or a partially suppressed reaction of the anterior lobe to estrone. In addition, certain further data concerning the action of testosterone compounds on the anterior lobe are presented which supplement and confirm previous observations (2). In these experiments the pituitaries were prepared for study and analyzed by methods previously described (3).

EXPERIMENTAL RESULTS

Series 1. Thirteen immature male and female rats (21 to 25 days of age) received daily iujections of 500 gamma of testosterone-propionate for 10 days. Eleven littermates were used as controls. No difference was found between the pituitary weights of the control and the experimental rats (table 1). Histologically, in the anterior pituitaries of the injected rats a great majority of the basophiles were degranulated. Cell counts revealed that the relative percentages of the granular basophiles were reduced to a mean far below that found in the

[1] These studies were aided by grants made to Vanderbilt University Medical School by the Grants-in-Aid Committee of the National Research Council and by the Division of Medical Sciences of the Rockefeller Foundation.
[2] Testosterone-propionate was furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. Testosterone-acetate was furnished by the Ciba Corporation through the courtesy of Mr. Robert Mautner.
[3] Theelin in oil was used. A portion of this material was furnished gratuitously by Parke-Davis and Company.

controls; inversely, the non-granular basophiles were increased (table 1). In both the granular and the non-granular basophiles the negative image of the Golgi apparatus was often prominent. In many partially degranulated basophiles small aggregates of coarse blue granules were observed. Careful focussing often revealed the presence of small orange bodies thought to be mitochondria. Constant or pronounced changes were not observed in the eosinophiles. For the most part these cells were well filled with granules; in many, the negative image of the Golgi apparatus was prominent. A very few of these cells did show evidence of loss of granules. The mean relative level of the eosinophiles in the injected rats was practically the same as in the controls (table 1). In the injected rats the chromophobes were not greatly changed from the normal, either histologically or quantitatively. A few were enlarged and presented an enlarged negative image of the Golgi apparatus. The mean

TABLE 1. SUMMARY TABLE GIVING THE MEAN (*M*) PERCENTAGES AND STANDARD DEVIATIONS (*SD*) OF CELLS IN THE PITUITARIES OF CONTROL RATS AND IN THOSE RECEIVING MALE HORMONE

Type of Rats	No. of Rats	Mean Pit. Weight mg.	Percentage of Cells				Mitoses per Section
			Eosin.	G. Baso.	N. G. Baso.	Chrom.	
Control	11	3.3	*M* 36.2 *SD* 3.5	*M* 6.1 *SD* 1.6	*M* 3.8 *SD* 1.3	*M* 53.9 *SD* 3.2	17
Male Hormone	13	3.2	*M* 37.6 *SD* 4.5	*M* 1.3 *SD* 0.7	*M* 5.9 *SD* 1.2	*M* 55.2 *SD* 3.7	9

relative level of these cells was practically the same as in the controls (table 1). Injection of the male hormone failed to increase the number of mitoses in the injected rats; rather they were more abundant in the controls. The results of these studies are in agreement with those made previously on the action of male sex factors on the anterior pituitary of the castrated female rat (2).

Series 2. Three groups of 10 young female rats weighing from 100 to 125 gm. were used. The first group received 10 daily injections of 500 γ of testosterone-acetate; the second group 10 daily injections of 500 γ of estrone, and the third group 10 daily injections of 500 γ of each of these two factors. Each 500 γ of hormone substance was dissolved in 0.16 cc. of peanut oil. The mean pituitary weight of the rats receiving only the male hormone was 6 mg. which is near the normal mean for rats of this weight. In the rats receiving estrone the mean pituitary weight was increased to 12.5 mg. while the mean pituitary weight of those which received both estrone and the male hormone was only 6.6 mg. (table 2). These data indicate that injection of the male hormone simultaneously with estrone almost completely suppressed the capacity of estrone to increase the weight of the hypophysis.

All anterior pituitaries were studied histologically. In those of rats receiving the male hormone alone, the mean level of the eosinophiles was 38.5 per cent (table 2) which is near the mean for these cells in normal female rats, i.e., 34.8 per cent (4). A great majority of these cells were well filled with granules; few showed evidence of loss of granules. The basophiles were

TABLE 2. FREQUENCY DISTRIBUTION TABLE GIVING LEVELS OF VARIOUS CELLS IN THE DIFFERENT GROUPS. THE MEAN (*M*) LEVELS OF CELLS AND THE MEAN PITUITARY WEIGHTS ARE ALSO GIVEN

Frequency Intervals in Percentages	Normal Female Rats			Castrated Female Rats			
	Male Hormone	Male Hormone & Estrone	Estrone	Control	Male Hormone	Male Hormone & Estrone	Estrone
Eosinophiles							
15–19.9			3				6
20–24.9			4				4
25–29.9			3			4	
30–34.9	4	2		6	5	6	
35–39.9	2	6		5	3		
40–44.9	4	2			1		
45–49.9					1		
Basophiles (G)							
0– .9	10	2	none		5		none
1– 1.9		5			2	3	
2– 3.9		3			3	6	
4– 5.9				1		1	
6– 7.9				9			
8– 9.9				1			
Baso. (N.G.)							
0– .9		1	4			2	7
1– 1.9		2	3	1		6	2
2– 3.9	3	7	3	10	5	2	1
4– 5.9	6				3		
6– 7.9	1				2		
Chromophobes							
45–49.9	2				1		
50–54.9	3	2		4	2		
55–59.9	1	4		7	2		
60–64.9	4	4			5	4	
65–69.9						5	
70–74.9			5			1	2
75–79.9			3				4
80–84.9			2				4
M Esionophiles	38.5	37.5	22.8	34.2	35.8	30.3	20.2
M Gran. Baso.	.5	1.6		7.1	1.4	2.4	
M Non. Gr. Baso.	4.5	2.1	1.3	2.8	4.8	1.8	.7
M Chromophobes	56.9	58.8	75.9	55.9	58.0	65.5	79.1
M Pit. Wt.	6.0	6.6	12.5	9.5	9.4	11.8	16.2

almost completely degranulated; the mean level of the granular basophiles was only 0.5 per cent. Non-granular basophiles were abundant (table 2). The chromophobes were normal, both histologically and quantitatively. These pituitaries were similar to those in series 1 which also received the male hormone.

The anterior pituitaries of those rats which received estrone alone (group 2) were morphologically and quantitatively different from those (group 3) which received both estrone and the male hormone. In rats receiving only estrone the basophiles were practically completely degranulated; only an occasional granular basophile was observed. In contrast, the pituitaries of those rats which received the male hormone in addition to the estrone contained

appreciable numbers of granular basophiles (table 2). Their occurrence, although limited, constituted a constant and definite distinguishing characteristic of the pituitaries of this group when they were compared with those of rats which received estrone alone. These cells were usually rather small; the negative image of the Golgi apparatus was prominent. In addition to the granules, small orange-colored bodies thought to be mitochondria were often observed. In both groups degranulated basophiles were abundant (table 2).

The pituitaries of rats which received only estrone contained numerous eosinophiles which showed evidence of loss of granules. Such cells were usually swollen and the negative image of the Golgi apparatus was usually increased in size. The eosinophiles were reduced in percentage to a mean level of 22.8 per cent, which is far below the level found in normal rats. In contrast, in the pituitaries of the rats which received male hormone in addition to estrone, a much smaller number of the eosinophiles showed evidence of loss of granules and the mean relative level of these cells in this group of pituitaries was 37.4 per cent which is well within the normal limits for normal female rats (4). In rats which received only estrone the chromophobes were greatly increased in relative percentage (table 2). Many of these cells were greatly enlarged; the cytoplasm was either dense blue or light blue in color and often fragmentary. In both the small and the enlarged chromophobes, the negative image of the Golgi apparatus was often hypertrophied. In many cells small orange-colored bodies, thought to be mitochondria, were observed. In those rats which received the male hormone in addition to estrone, the chromophobes were not increased in relative percentage over that level found in normal rats (table 2). Histologic changes were markedly less noteworthy than in those rats which received estrone alone. Analysis of these data indicate that in normal female rats injection of testosterone-acetate simultaneously with estrone partially suppressed the capacity of estrone to induce the characteristic changes in the anterior lobe that are brought about by the administration of this estrogenic agent alone.

Series 3. The experiments described in series 2 were repeated, using castrated female rats. Three groups of 10 rats received, respectively, 10 daily injections of 500 γ of testosterone-acetate, 10 daily injections of estrone, and 10 daily injections of 500 γ each of estrone and testosterone-acetate. A fourth group of 10 non-treated castrated female rats were used as controls. Injections of the male hormone failed to alter the pituitary weights of the injected rats from those of the controls; injections of estrone induced a marked weight increase in the pituitary and injections of testosterone-acetate simultaneously with estrone supressed the capacity of estrone to increase greatly the pituitary weights of those rats which received the combined injections (table 2).

In the 10-day castrate controls, the usual post-castration changes were observed; the basophiles were increased in size and relative numbers (table 2). A majority contained granules although some were degranulated. Injections of the male hormone prevented such castration changes in the basophiles; those present after injection were mostly degranulated. The relative

levels of the eosinophiles in the injected rats were practically the same as in the controls (table 2). No noteworthy histologic changes were induced in either the eosinophiles or chromophobes by injection of the male factor.

In the pituitaries of rats receiving estrone the castration-increase in the basophiles was not only prevented but the degranulation of the few basophiles present was complete (table 2). However, in the pituitaries of those rats which received the male hormone in addition to estrone, considerable numbers of granular basophiles were found (table 2). Degranulated forms were found in both groups. In the pituitaries of rats receiving only estrone the relative mean level of the eosinophiles was reduced to 20.2 per cent; the mean level in the castrate controls was 34.2 per cent (table 2). Numerous individual cells showed evidence of loss of granules. In rats receiving both

TABLE 3. SUMMARY TABLE GIVING MEAN (*M*) PERCENTAGES AND STANDARD DEVIATIONS (*SD*) OF CELLS IN PITUITARIES OF CONTROL MALE RATS AND IN THOSE RECEIVING MALE SEX HORMONE, ESTRONE AND COMBINATIONS OF MALE SEX HORMONE AND ESTRONE

Type of Rats	No. of Rats	Mean Pit. Weight mg.	Percentages of Cells			
			Eosin.	G. Baso.	N.G. Baso.	Chrom.
Control	30	6	*M* 48.5 *SD* 5.8	*M* 5.4 *SD* 1.8	*M* 1.0 *SD* 0.3	*M* 45.1 *SD* 5.9
Male Hormone	6	5.7	*M* 48.1 *SD* 4.2	*M* 1.8 *SD* 1.4	*M* 2.8 *SD* 1.0	*M* 44.3 *SD* 5.5
Male Hormone Estrone	6	7.3	*M* 46.6 *SD* 4.1	*M* 3.2 *SD* 0.8	*M* 2.7 *SD* 1.5	*M* 47.5 *SD* 4.4
Estrone	12	10.7	*M* 35.0 *SD* 4.4	*M* 0.5 *SD* 0.2	*M* 2.4 *SD* 1.5	*M* 62.1 *SD* 4.96

the male hormone and estrone, the mean relative level of the eosinophiles was 30.5 per cent, only a slight reduction below the mean level of these cells in the controls (table 2). Relatively few eosinophiles showed loss of granules. In the anterior lobes of rats receiving only estrone, the chromophobes were markedly increased in percentage to a mean level of 79.1; the mean level in the controls was 55.9 per cent. In the estrone-injected rats a tremendous number of the chromophobes were greatly enlarged; in many the negative image of the Golgi apparatus was hypertrophied. In some instances the cytoplasm was dense blue in color; in others it was light blue and fragmentary. In rats receiving the male hormone in addition to estrone, the mean level of the chromophobes was increased to 67 per cent, a considerable increase over the level of these cells in the castrated controls but not nearly so marked as that found in the rats which received only estrone (table 2). The histologic changes were of a lesser intensity than those found in the chromophobes of the pituitaries of the rats which received only estrone. These data indicate that injection of the male hormone simultaneously with estrone partially suppressed the reaction of the anterior lobe to the administration of estrone in castrated female rats as well as in non-castrated animals; there is some indication, however, that this suppression was not as marked.

Series 4. The same type of experiment was repeated on normal male rats. Three groups of 6 each received, respectively, 10 daily injections of 500 γ of testosterone-propionate, 10 daily injections of 500 γ of estrone, and 10 daily injections of 500 γ each of these two factors. Thirty normal rats were used as controls. The pituitary weights of those rats which received only the male hormone were practically the same as those of the controls, those of the rats which received only estrone were markedly increased over those of the controls while those of rats which received both the male hormone and estrone were only slightly greater than those of the controls.

In rats receiving only the male hormone a great majority of the basophiles were degranulated; non-granular basophiles were abundant (table 3). The relative levels of the eosinophiles were as high as those in the controls (table 3). No marked histologic changes were observed in the eosinophiles. The chromophobes appeared normal, both histologically and quantitatively.

In the pituitaries of the rats receiving only estrone, the eosinophiles were markedly reduced in relative percentage when compared with the level of these cells in the controls (table 3). Numerous individual cells showed evidence of loss of granules. In rats receiving the male factor in addition to estrone, the relative percentages of the eosinophiles were practically the same as in the controls and as in the rats which received only the male hormone (table 3). Relatively few cells showed evidence of loss of granules. The basophiles in the pituitaries of rats receiving only estrone were almost completely degranulated, while in the pituitaries of those rats which received both estrone and the male hormone, granular basophiles were much more abundant (table 3). In the pituitaries of rats receiving only estrone the chromophobes were markedly increased in percentage (table 3). The same marked histologic changes were observed as in the chromophobes of other groups which had received only estrone (series 2 and 3). In those rats receiving the male hormone in addition to estrone, the chromophobes were not increased in percentage and histologic changes were not as marked as those observed in the estrone group. These data indicate that in male as well as in female rats, injection of the male hormone simultaneously with estrone, reduces the capacity of estrone to induce characteristic changes in the anterior pituitary.

DISCUSSION

These studies present evidence that the action of the male hormone on the anterior pituitary differs from that of estrone. Injection of estrone resulted in weight increase of the gland, marked degranulation of the basophiles, degranulation of some of the eosinophiles and a decrease in the relative percentages of the granular cells with a corresponding increase in those of the non-granular. The only constant and noteworthy effect of the injections of male hormone in this series seemed to be degranulation of the basophiles. However, there is the possibility that injection of larger amounts of male hormone would have resulted in more marked pituitary changes.

These studies further indicate that in either normal or castrated female

rats or in normal males, injection of the male hormone simultaneously with estrone, either partially suppressed the capacity of estrone to induce a weight increase and characteristic histologic changes in the anterior lobe or else restricted the capacity of the anterior lobe to react to the injected estrin in a characteristic manner. Zuckerman (5) has reported that certain male sex factors restricted the capacity of estrin to induce certain changes in the prostate of the monkey while Robson (6) has reported that testosterone, injected simultaneously with estrone, inhibited the capacity of the estrone to induce vaginal cornification in the castrated mouse. These findings indicate that the male hormone possesses the capacity to restrict the action of estrone on several tissues.

As previously pointed out (1), injection of large amounts of APL simultaneously with estradiol, partially suppressed the capacity of this estrogenic agent to induce its usual effects on the anterior lobes of male and female rats with intact gonads; such results were not obtained in castrated rats. In these experiments it seems that the APL must have exerted its suppressing effects on the action of estradiol on the anterior lobe by its action on the gonads. In the experiments using the non-castrated males it seems probable that the APL stimulated the production of the male hormone in the injected rat and that this factor was responsible for the suppression of the action of estradiol on the anterior lobe, in the same manner that injected male hormone induces the same suppression. In the experiments on the non-castrated females a suitable interpretation is more difficult. However, our data indicate that injection of large amounts of APL, simultaneously with estradiol, stimulated the production of some factor in the ovary which partially suppressed the action of estradiol on the anterior lobe in the same manner as does the male hormone. Whether this factor was actually the male hormone or some related substance is at present uncertain. Support of the hypothesis that the ovary under certain conditions may produce a male-like hormone is found in the recent reports that ovaries, transplanted into male mice, maintained the accessory reproductive organs (7) (8). It has also been reported that normal and pregnant women excreted the male hormone in the urine (9), (10), (11), (12). Other studies (13), (14) indicate that the paraurethral glands or Skene's ducts, a homologue of the male prostate, found in the female are under the control of the male hormone and not of the female hormone. Since they are occasionally well developed in the normal female rat, their presence might present further evidence that the ovaries under certain conditions secrete a factor which exhibits certain of the physiologic properties of the male hermone.

SUMMARY

Thirteen immature rats received 10 daily injections of 500 γ of testosterone propionate. Eleven littermates served as controls. Such injections induced degranulation in the basophiles. No noteworthy changes were observed in the eosinophiles or the chromophobes. Mitotic activity was not increased in the experimental rats; the pituitary weights were unaltered from those of the controls.

Three groups of 10 female rats received, respectively, 10 daily injections of 500 γ of testosterone-acetate, 10 daily injections of estrone, and 10 daily injections of 500 γ each of both these factors. Injections of the male factor simultaneously with estrone, partially suppressed the characteristic reaction of the anterior lobe to injection of estrone, i.e., marked weight increase, complete degranulation of the basophiles, degranulation of numerous eosinophiles and a marked decrease in the percentages of the chromophilic cells and an increase in those of the chromophobes. A similar suppression of the action of estrone on the anterior lobe by injection of male hormone simultaneously with estrone, was also obtained in similar groups of castrated female rats and normal males.

Cell counts were made on all pituitaries studied; the resulting quantitative data were presented statistically.

The possibility that the ovaries elaborate some factor possessing certain of the properties of the male hormone is discussed.

REFERENCES

1. Wolfe, J. M.: Anat. Rec. **68:** 237. 1937.
2. Wolfe, J. M., and J. B. Hamilton: Anat. Rec. **67:** 55. (Suppl.) 1937.
3. Wolfe, J. M., and C. S. Chadwick: Endocrinology **20:** 503. 1936.
4. Wolfe, J. M.: Anat. Rec. **61:** 321. 1935.
5. Zuckerman, S.: Lancet **1:** 1259. 1936.
6. Robson, J. M.: Proc. Soc. Exper. Biol. & Med. **35:** 49. 1936.
7. Hill, R. T., and W. U. Gardner: Anat. Rec. **64:** 21. (Suppl.) 1936.
8. Hill, R. T.: Anat. Rec. **67:** 24. (Suppl.) 1937.
9. Womack, E. B., and F. C. Koch: Endocrinology **16:** 273. 1932.
10. Loewe, S., H. E. Voss and E. Rothschild: Biochem. Ztschr. **237:** 214. 1931.
11. Kabak, J. M.: Endokrinologie **10:** 12. 2.
12. Siebke, H.: Arch. f. Gynäk. **56:** 317. 1934.
13. Korenchevsky, V., and M. Dennison: J. Path. & Bact. **42:** 91. 1936.
14. Hamilton, J. B., and J. M. Wolfe: Proc. Soc. Exper. Biol. & Med. (In press).

THE SPERMATOGENIC AND SECRETORY FUNCTION OF THE GONADS OF HYPOPHYSECTOMIZED ADULT RATS TREATED WITH PITUITARY FSH AND LH

ROY O. GREEP AND HARRY L. FEVOLD
From the Biological Laboratories, Harvard University[1]
CAMBRIDGE, MASSACHUSETTS

In a previous report (1) we demonstrated a marked structural and functional difference in the response of immature male rat gonads to injections of two hypophyseal gonadotropic fractions. The FSH fraction which is distinguished by its ability to develop Graafian follicles in the mammalian ovary showed a similar specificity of action in immature males by stimulating the seminiferous epithelium without causing hypertrophy of the Leydig cells. The luteinizing preparation (LH), however, produced only hypertrophy of the interstitial tissue and, as a consequence, the secondary sexual structures were greatly enlarged. These studies clearly indicated that the same secretions of the hypophysis which in the female promoted the maturation of follicles and their subsequent conversion into corpora lutea have equally diverse actions on the male gonad. The presence of the LH so often noted in the pituitary gland of the males of several sepecies and of the FSH as well was given a physiological interpretation.

The present report extends these observations to the hypophysectomized sexually mature male rat. The experiments are of two categories; those in which treatment was started immediately after hypophysectomy and those in which treatment was postponed for sufficient time to allow considerable regression of the entire reproductive system. The males were young animals weighing 220 gm. at the time of operation.

IMMEDIATE TREATMENT

When treatment with either FSH or LH was started shortly after hypophysectomy and continued for 40 days the testes did not ascend into the abdomen nor undergo appreciable changes in size. There was, however, a tendency for the testes to become slightly flaccid (palpation) during the early part of treatment with either fraction. The normal color and oedema of the scrotum was retained only with LH therapy. Spontaneous emission of ejaculate was frequetly noticed in LH-treated males but rarely occurred in the group receiving FSH and then only during the first days of treatment.

Matings were not attempted as it was felt that the state of cachexia would influence the libido sexualis to the extent that a failure to mate would not accurately reflect their propensities in that direction. It may be stated as a

[1] Aided by a Grant from the Rockefeller Foundation administered by Professor Frederick L. Hisaw.

TABLE 1. FSH OR LH INJECTED INTO HYPOPHYSECTOMIZED ADULT MALE RATS. IMMEDIATE TREATMENT

Material	Daily dosage	Time	Testes	Seminal vesicles
		days	mg.	mg.
Normal controls			2691[1]	670
Hypophysectomized 15 days			1289	88
Hypophysectomized 30 days			500	61
FSH (G6)	1 R.U.	15	2300[1]	102
" (G5)	1/12 R.U.	15	2515	105
" (G5)	1/4 R.U.	20	2100	130
" (G5)	1/12 R.U.	30	2050	156
" (G6)	1 R.U.	40	1770	178
" (G5)	1/12 R.U.	40	2100	120
LH (GL6)	1 mg.[2]	15	2538	655
" "	2.5 mg.	15	2520	1120
" "	5 mg.	15	2865	1595
" "	1 mg.	40	2190	240
" "	2.5 mg.	40	1957	220
" "	5 mg.	40	2380	310

[1] The autopsy weights are averages of 2 animals in each experiment and 3 in each untreated group.
[2] 15 mg. of LH preparation GL6 is the equivalent of 1 gm. sheep pituitary powder.

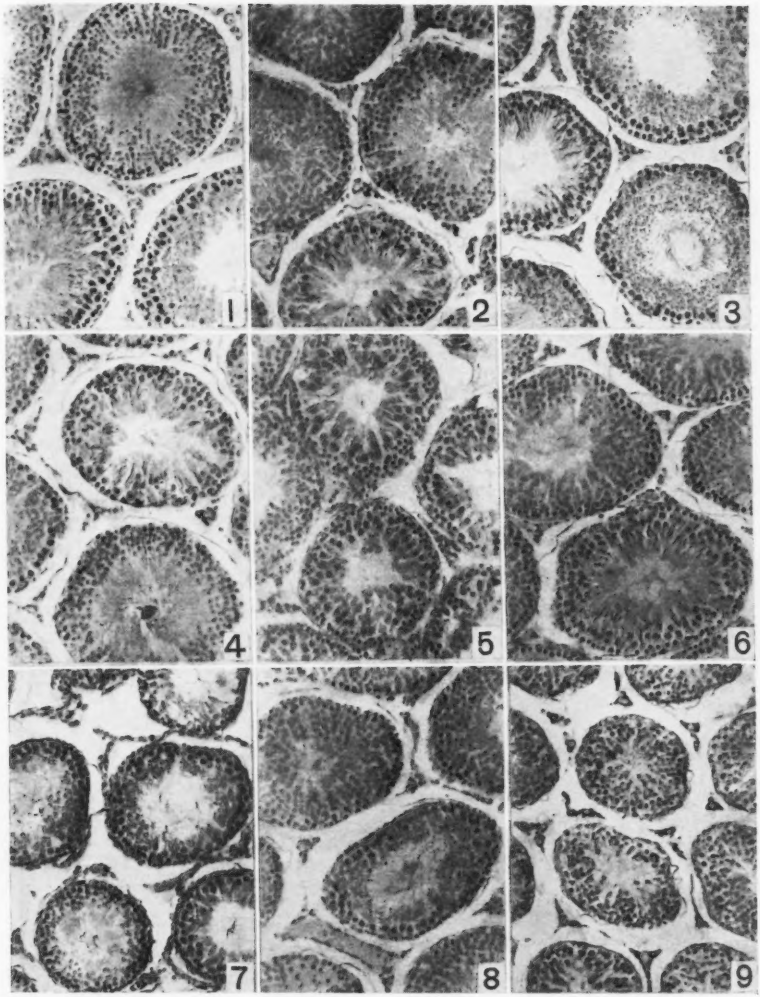
general observation that in all of our work the FSH has proven to be entirely devoid of ability to alleviate the symptoms of hypopituitarism which become progressively severe after removal of the gland. The LH fraction, however, has decided beneficial body effects and even though the adrenals and thyroids may show no appreciable increase in size over their untreated operated controls it is likely that they received mild stimulation from contaminating traces of adrenotropic and thyrotropic principles. The possibility that the secretions of the testes, especially with LH treatment, may be beneficial in alleviating the cachexia were negated by finding that the injection of testosterone in high doses was ineffectual in this regard.

Injections of FSH in sufficient doses to sustain normal gonad size for 30 days after hypophysectomy allowed regression within 15 days of all those sexual

TABLE 2. FSH OR LH INJECTED INTO HYPOPHYSECTOMIZED ADULT MALE RATS. TREATMENT POSTPONED 15 DAYS

Material	Daily dosage	Time	Testes	Seminal vesicles
		days	mg.	mg.
Normal controls			2691[1]	670
Hypophysectomized 15 days			1289	88
Hypophysectomized 30 days			500	61
FSH (G5)	1 R.U.	7	1400[1]	150
" (G6)	8 R.U.	7	1300	184
" (G5)	1/6 R.U.	15	1920	396
" (G6)	8 R.U.	15	1620	140
" (G5)	1/6 R.U.	30	2310	161
" (G5)	1/3 R.U.+0.18 mg. testosterone	15	1590	1540
LH (GL6)[2]	2.5 mg. (pwd.)	15	900	320
" "	5 mg. (pwd.)	15	1520	1420
" "	2.5 mg. (pwd.)	30	1220	175
" "	5 mg. (pwd.)	30	1635	720

[1] The autopsy weights are averages of 2 animals in each experiment and 3 in each untreated group.
[2] 15 mg. of LH preparation GL6 is the equivalent of 1 gm. sheep pituitary powder.



Figs. 1-9. *1*. Section of testis of normal control male. *2*. Testis of hypophysectomized adult rat given 1 R.U. FSH daily for 40 days. Injections started at time of operation. x 145. *3*. Testis of hypophysectomized adult rat given 5 mg. LH daily for 15 days. Injections started at time of operation. x 145. *4*. Testis of hypophysectomized adult rat given 5 mg. LH daily for 40 days. x 145. *5*. Testis of hypophysectomized adult rat given 1 R.U. FSH daily for 7 days. Injections started 15 days after operation. x 145. *6*. Testis of hypophysectomized adult rat given 1/6 R.U. FSH daily for 30 days. Injections started 15 days after operation. x 145. *7*. Testis of hypophysectomized adult rat given 5 mg. LH daily for 15 days. Injections started 15 days after operation. x 145. *8*. Testis of hypophysectomized adult rat given 5 mg. LH daily for 30 days. Injections started 15 days after operation. x 145. *9*. Testis of adult rat hypophysectomized for 15 days. x 145.

structures dependent upon gonadal secretions (table 1 and fig. 10). Treatment lasting 40 days showed a slight loss in weight of the testes which was attributed in part at least to the debilities resulting from pituitary deprivation. Sperm were found in the epididymes of all FSH-treated animals at autopsy and histological examination of the testes showed that sperm were continually

being produced and liberated into the lumen of the tubles (fig. 2). The interstitual cells were characteristic of those of untreated hypophysectomized controls in having densely stained nuclei with reduced amounts of cytoplasm. Doses of FSH in excess of that necessary for maintaining the testes did not produce gonads of greater than normal size.

Injections of LH sustained the full reproductive complement without qualification for at least 15 days. With longer periods of treatment (30 to 40 days) the seminal vesicles were always smaller than they had been at 15 days for any given dosage level (table1). Verification of an initial intense stimulation of the interstitial cells followed by subsequent regression at 30 and 40 days of treatment was found by microscopic examination of these gonads (figs. 3 and 4). Gametogenesis was not impaired in any way by the LH even

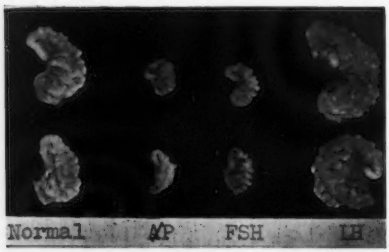


Fig. 10. Showing the size of the seminal vesicles of hypophysectomized adult rats after 15 days of immediate treatment with FSH or LH as compared with those of normal and untreated operated control animals.

in experiments lasting 40 days after the hypophysis had been removed (fig. 4). The maintenance dosage for the secondary sexual structures was surprisingly low (the equivalent of 1/15 gm. per day of sheep pituitary powder). Larger doses of LH produced a somewhat proportional increase in the size of the accessories over those of untreated normal animals of comparable age and weight (fig. 10).

POSTPONED TREATMENT

In testing the reparative action of FSH or LH the treatments were uniformly started 15 days after removal of the pituitary gland. At this time the seminiferous tubles were greatly reduced in diameter and showed advanced cellular involution (fig. 9). The spermatids though highly vacuolated were yet recognizable and in 1 of 4 animals a few sperm were yet present. The testicles occupied a position in the upper portion of the inguinal canal and the scrotum had largely disappeared.

Injections of FSH effected a gradual restoration of the atrophic tubules which culminated in their return to active spermatogenesis (figs. 5 and 6). The histological picture of testes secured after 7 days FSH stimulation showed an unusual predominance of spermatids with a few sperm heads present deep down in the cellular wall in some tubules. Mature sperm were not found at this time (22 days postoperative), the lumina of the tubules were empty and

the epididymes likewise were entirely free of sperm. The seminal vesicles showed either slight or no stimulation (table 2). After 15 days of FSH therapy the tubules contained mature sperm. These were abundant in the lumina of the tubules and had by this time migrated into the tail of the epididymes. The seminal vesicles were for the most part slightly heavier than those in untreated operated controls. The dosage of FSH used in this series of animals was probably greater than was essential for complete restoration of the tubules and it was assumed that whatever increase in seminal vesicle size occurred was due to contamination of the FSH with traces of LH so that with increased dosage the LH reached a sufficient concentration to produce some interstitial cell activity.

The original preoperative testicular size was likewise gradually regained within 30 days of FSH treatment (table 2). It appeared that the factor of time was much more important than dosage in effecting a regrowth of the testes since the response was always about the same for any given period of treatment regardless of a twelve-fold variation in dosage. The testes slowly returned to the completely scrotal position after beginning the FSH treatment and remained there throughout the experimental period. The scrotum underwent sufficient development to accommodate the enlarged gonads but remained pale and non-edematous.

Repair of the gonads following LH action was slower and less pronounced than with FSH. After 15 days of treatment (fig. 7) with the 5 mg. daily dose of LH a few mature sperm were present while half that dosage produced only a few sperm heads in a few tubules. By the 30th day mature sperm were found in all instances both in the tubules (fig. 8) and in the tail of the epididymes. With the lower doses of LH the weights of the testes showed merely a retardation in the rate of regression while with higher doses they showd a significant increase after 15 days of treatment and still further improvement after 30 days (table 2). It is of interest, however, that the LH in no instance ever restored the testes to their original magnitude as did the FSH. The soft and spongy character of the testes following 15 days of postoperative atrophy was not greatly altered by subsequent LH treatment. The testes reassumed the scrotal position soon after the treatment was begun. Complete repair of the reproductive accessories was effected by the LH[2] and the size of the seminal vesicles after 15 days of treatment was a fair expression of the dosage employed. At the end of 30 days of reparative treatment we again found, as in those animals treated immediately after operation, that the accessories were smaller for each dosage level than was noted at 15 days. The histological findings likewise were similar in showing hypertrophy of the Leydig cells after 15 days of treatment and definite cellular depletion after 30 days (figs. 7 and 8).

DISCUSSION

The influence of these gonadtropic fractions of the anterior lobe of the hypophysis on spermatogensis in adult rats is of special significance in view

[2] In a recent report Evans, Korpi, Simpson, Pencharz and Wonder (Univ. California Press 1:255, 1937) have contended that the interstitial tissue stimulating ability of the hypophysis which we have attributed to the LH substance is due to a separate pituitary principle. If this be true the LH preparation used in these experiments must contain both the interstitial cell-stimulating and luteinizing principles.

of the current trends in the endocrinology of the male reproductive system. The experiments of Walsh, Cuyler and McCullagh (2) showing that gametogenesis can be maintained in the adult rat by male sex hormone after hypophysectomy have been adequately confirmed and extended by Nelson (3, 4). The major implications of these results argue for the dispensability of a pituitary factor in sperm formation and suggest that in the normal animal an androgenic stimulus would be adequate for maintenance of the entire reproductive system. It cannot be denied, however, that injected FSH has a selective action on the germinal elements of both the male and female gonads. Furthermore, the ability of either the male or female hypophysis to simulate the effects of injected FSH in the sense of growing follicles has been convincingly demonstrated by homoplastic pituitary implants (5, 6) and by the parabiosis studies of Witschi and Levine (7). These facts prove that the male pituitary normally secretes FSH. As a matter of opinion it seems illogical to assume that in the normal economy of the male organism this substance is vestigial in character. The presence of LH in the hypophysis of male rats is likewise established by finding extensive luteinization in the ovaries of hypyphysectomized immature female rats treated with acetone dried glands (8).

By the experiments herein reported it is shown that FSH alone is able not only to sustain gametogenic processes in the absence of testicular secretion (as evidenced by atrophic accessories) but also to restore the tubules to sperm-forming organs without the aid of sex hormones once this function has been lost through induced atrophy. The slight enlargement of the seminal vesicles in animals receiving FSH as a 'reparative' treatment (table 2) is evidence that a small amount of male sex hormone was being produced. That the observed tubular repair was not appreciably influenced by this amount of male hormone was indicated by the following observations: *1)* The repair was not correlated in any way with seminal vesicle size. *2)* The addition of testesterone to the FSH in sufficient dosage to produce seminal vesicles twice the size of those of normal control rats did not enhance the effectiveness of the FSH as regards spermatogenesis or testicular growth. *3)* When FSH was given immediately after hypophysectomy spermatogenesis was maintained even though the accessories underwent regression.

We have reported the ability of an extract of Brewer's Yeast (10) to induce testicular hypertrophy in young male rats and to sustain gametogenesis in the adults after removal of the pituitary gland, and Nelson (4) has reported that the progestational hormone of the corpus luteum also will maintain spermatogenesis under similar conditions. The influence of these substances as well as of male hormone on sperm formation in the rat is limited, however, in that they are ineffectual as reparative agents if a short period of post-operative atrophy is allowed.

In this connection the ability of LH to bring about the reappearance of sperm in testes that have been deprived of pituitary stimuli for 15 days was unexpected. One of the possibilities in this regard was that the LH may contain traces of FSH which aided in the recovery of the tubules. This possibility while not conclusively eliminated was rendered unlikely in view of the fact

that the LH was tested on hypophysectomized immature female rats in total dosages (over 5 days) exceeding by 50 times the minimal effective daily dose for maintaining the male accessories and it did not stimulate the growth of follicles, the uteri remained infantile and the vaginae did not open.

Smith and Leonard (11) have shown that the gonad-stimulating principle in pregnancy urine has a decided beneficial effect on the maintenance of reproductive function in hypophysectomized sexually mature male rats when the treatment was started immediately after operation. Even when the treatment was delayed for 32 days a sufficient repair was induced to cause the reappearance of spermatids after 21 days. It seems probable that these effects were due in part to an indirect stimulation of the tubules by the hormone of the interstitial gland secreted in response to the luteinizing property of pregnancy urine. The stimulatory effects of PU and of pituitary LH on atrophic tubules, whether direct or indirect are comparable with the exception that LH carried the spermatids through their final maturation into spermatozoa. Smith and Leonard also stated that PU produced at first a stimulation of the interstitial tissue (and enlarged accessories) in hypophysectomized rats which was then followed by involution of the interstitial elements and a reduction in size of the accessories by the 30th day of treatment. These observations are in concordance with the initial stimulation and terminal regressive changes observed in the interstitial cells and accessory structures when LH was administered for a similar period. On the 40th day of LH treatment these regressive changes were much more pronounced. The reason for this failure of the interstitial tissue after prolonged treatment is not clear but we presume that it is due either to secretory exhaustion or to the development of a physiological immunity against the hormone stimulating this tissue.

Empiricially it has been shown that as far as the seminiferous tubules in recently hypophysectomized adult rats are concerned the end result is the same with either FSH or LH therapy, i.e., sperm production goes on unabated. But, since we now have evidence from many sources (12, 13, 14, 15, 16, 17, 18, 9, 1) to believe that these two pituitary fractions represent two hypophyseal products with different chemical and physiological properties and that this fractionation has been carried to a reasonable degree of completeness we have concluded on the basis of all the evidence available that the mechanism of tubular stimulation is not the same for these two fractions. The FSH effect is definitely direct while with LH the tubules appear to utilize the androgens produced in response to the LH and in some instances (as after a period of atrophy) they may also use either the LH or some other pituitary protein associated with this fraction.

SUMMARY

When FSH was administered to sexually mature male rats immediately following the ablation of the hypophysis no interruption of sperm formation was noted within 40 days of treatment. The accessory structures, however, underwent early regression showing that the secretion of the male hormone had not been prolonged by the FSH after hypophysectomy. The maintenance

of tubular structure by FSH, therefore, was not dependent in any way upon the presence of male hormone. When treatment was delayed 15 days post-operative the atrophic tubules were restored to full spermatogenesis. This repair was not different in the presence or absence of male hormone.

Injections of LH immediately following hypophysectomy sustained both the gametogenic and endocrine activities of the adult testes for a time. Partial regression of the accessories occurred after 30 to 40 days of treatment but spermatogenesis was not impaired up to 40 days at least. When LH was given after a 15-day period of atrophy interstitial cell repair was rapid and sperm formation was eventually resumed. The mechanism by which the LH is able to sustain or cause the reappearance of spermatogenesis in hypophysectomized adult rats is not clear.

REFERENCES

1. Greep, R. O., H. L. Fevold and F. L. Hisaw: Anat. Rec. **65:** 261. 1936.
2. Walsh, E. L., W. K. Cuyler and D. R. McCullagh: Am. J. Physiol. **107:** 508. 1934.
3. Nelson, W. O., and T. F. Gallagher: Science **84:** 230. 1936.
4. Nelson, W. O.: Anat. Rec. **67:** Suppl. 36. 1937.
5. Evans, H. M., and M. E. Simpson: Am. J. Physiol. **89:** 371. 1929.
6. Smith, P. E.: Proc. Soc. Exper. Biol. & Med. **24:** 131. 1926.
7. Witschi, E., and W. T. Levine: Proc. Soc. Exper. Biol. & Med. **32:** 101. 1934.
8. Hellbaum, A. A., and R. O. Greep: Unpublished data.
9. Lipschütz, A.: Quart. J. Exper. Physiol. **25:** 111. 1935.
10. Hisaw, F. L., R. O. Greep and H. L. Fevold: Anat Rec. **67:** Suppl 50. 1936.
11. Smith, P. E., and S. L. Leonard: Anat. Rec. **58:** 145. 1934.
12. Fevold, H. L., F. L. Hisaw, A. Hellbaum and R. Hertz: Am. J. Physiol. **104:** 710. 1933.
13. Fevold, H. L., and F. L. Hisaw: Am. J. Physiol. **109:** 655. 1934.
14. Wallen-Lawrence, Z.: J. Pharmacol. & Exper. Therap. **51:** 263. 1934.
15. Foster, M. A., and F. L. Hisaw: Anat. Rec. **62:** 75. 1935.
16. Hisaw, F. L., R. O. Greep and H. L. Fevold: Anat. Rec. **61:** Suppl. 24. 1935.
17. Witschi, E., and C. A. Pfeiffer: Anat. Rec. **64:** 85. 1935.

THE QUANTITATIVE ASSAY OF "FOLLICLE STIMULATING" SUBSTANCES[1]

LOUIS LEVIN[2] AND H. H. TYNDALE
From the Department of Anatomy, College of Physicians and Surgeons, Columbia University
NEW YORK CITY

A number of the responses elicited in immature female rats and mice by gonadotropic extracts prepared from various sources have been applied to the quantitative assay of these extracts. The production of follicular enlargement, luteinization, 'blood point' formation, increase in ovarian weight, precocious vaginal canalization and induction of estrus as judged by the vaginal smear have all been used, singly or in combination, as indicators of the gonadotropic potency of the injected material.

At the inception of our work on the concentration and purification of the urinary gonadotropic material of ovariectomized and post-menopausal women, it became imperative to assay our preparations as accurately as possible. Earlier work (1) demonstrated that this gonadotropin is predominantly follicle-stimulating in nature, thus precluding the possibility of using luteinization or hemorrhagic follicle formation as a criterion of activity. Attempts were therefore made to assay the preparations, using immature mice, on the basis of follicular enlargement, increase in ovarian weight and vaginal canalization. In addition, since the uteri of the test animals were much enlarged in every positive test, this organ was routinely weighed.

The results of such experiments soon showed that the weight of the uterus is a most sensitive indicator of the potency of the preparation being tested and that by using this as a criterion of induced ovarian activity much more accurate and reproducible assays can be obtained than by use of any combination of the other three responses studied. Since the increase in uterine weight is the result of a functional response of the ovary, i.e., increased estrin secretion, it appears to be of greater physiological significance than does the simple increase in ovarian weight, and consequently seems a more logical test of the potency of extracts which are predominately follicle stimulating. Therefore, experiments were set up to demonstrate the effect of follicle-stimulating substances on the ovarian and the uterine weight and to point out that the uterine response as determined by its weight is a much more sensitive and constant indicator than is the ovarian response per se. The results of such studies are presented in this communication.

EXPERIMENTAL

Methods. Mice from our own highly inbred colony (Bagg albinos) were used as experimental animals, treatment beginning on the 21st to 23rd day of

[1] Aided by a Grant, administered by Dr. P. E. Smith, from the Committee for Research in Sex, The National Research Council.
A preliminary report of this work was given before the forty-ninth annual meeting of the American Physiological Society at Memphis, April 22, 1937.
[2] E. R. Squibb and Sons Fellow.

life. At this age these mice weigh from 7 to 10 gm. A total of 563 mice were used in the experiments reported in this paper. However, additional confirmative data, not reported here, have been obtained from routine assays employing some 8,000 mice over a period of 3 years.

Gonadotropic material was administered subcutaneously once daily on 3 consecutive days and the animals were sacrificed on the fourth day, i.e., 72 to 76 hours after the first injection. The reasons for this relatively early autopsy will be discussed in a later section of this paper. Just prior to autopsy the vaginal orifice was examined and graded as closed, swollen or open. At autopsy

TABLE 1. RELATION OF TIME TO RESPONSE OF MOUSE OVARY AND UTERUS TO CU (L411)

Total Dose Dry Powder, mg.			0.12	0.24	0.36	0.60
Ovarian Response						
72 hours[1]	Weight	mg.	5.4	6.1	7.3	9.1
	Increase[2]	mg.	0.7	1.4	2.6	4.4
96 hours[1]	Weight	mg.	5.1	5.5	6.2	7.4
	Increase[2]	mg.	0.4	0.8	1.5	2.7
Ratio:	72 Hr. Increase / 96 Hr. Increase		1.75	1.75	1.73	1.63
Uterine Response						
72 hours[1]	Weight	mg.	14.6	36.4	44.7	38.1
	Increase[2]	mg.	8.7	30.5	38.8	32.2
96 hours[1]	Weight	mg.	11.2	23.2	25.6	30.2
	Increase[2]	mg.	5.3	17.3	19.7	24.3
Ratio:	72 Hr. Increase / 96 Hr. Increase		1.64	1.76	1.97	1.33

[1] Autopsy performed at designated time after first injection; 15 mice in each 72-hour group; 8 mice in each 96-hour group.
[2] Increase over control weight, 33 mice; Ovary 4.7 mg., uterus 5.9 mg.

the ovaries were examined for follicular size and for luteinization under the dissecting microscope and were weighed on an air-damped analytical balance. At the same time, the uterus was removed,[3] stripped of its mesentery (a simpler and easier process than dissecting out the ovaries), freed of intra-uterine fluid by pressure against a piece of dry filter paper and weighed. This whole process requires but 1 to 2 minutes per animal.

The post-menopausal (or castrate) gonadotropic material was prepared from urine by our tannic acid precipitation method and was subsequently purified to a considerable extent (2). The response obtained in mice with this product does not differ from that obtained with the crude tannate or the native urine. The absence of estrogenic substances was proven by injection into

[3] The ovaries, fallopian tubes and uterus are removed at one operation by cutting at the utero-cervical junction with iridectomy scissors and lifting the entire tract out. The ovaries are dissected out and the mesentery then stripped from the body of the uterus by tension with fine forceps. Finally the fallopian tubes are removed by cutting at the points of junction with the uterus.

spayed rats as well as by the fact that all potency was destroyed by heating, acid treatment, etc., procedures which would not injure estrin if present.

RESULTS

Time of Autopsy. Maximal response and shortest possible assay time are of great importance in any assay method. A study of ovarian and uterine response to CU[4] showed that the routine Aschheim-Zondek technique (3) does not produce optimum effects.

Groups of mice were injected with CU over 3 days, receiving 1 injection daily. Some of the mice were autopsied 72 hours after the first injection while others were sacrificed 24 hours later (96 to 100 hours after the first injection)

TABLE 2. RESPONSE OF MOUSE OVARY AND UTERUS TO CU. (L411)

Total Dose L411	No.[1] Mice	Mean Body Wt.	Ovaries		Uterus	
			Weight Mean $\pm \epsilon_M$[2]	Increase over Control	Weight Mean $\pm \epsilon_M$	Increase over Control
mg.		gm.	mg.	per cent	mg.	per cent
0.00	33	9.9	4.7±0.1	—	5.9±0.3	—
0.09	15	10.7	5.0±0.1	6	9.9±0.5	68
0.12	16	10.9	5.4±0.2	15	14.6±1.0	147
0.16	15	10.9	5.5±0.2	17	20.2±1.1	242
0.20	15	11.3	5.7±0.2	21	24.3±1.8	312
0.24	15	11.3	6.1±0.2	30	36.4±1.1	517
0.36	15	12.0	7.3±0.3	55	44.7±1.6	658
0.60	14	11.3	9.1±0.3	94	38.1±1.3	546
1.20	15	11.5	15.0±0.5	219	36.5±1.3	519
1.80	15	11.2	18.9±0.6	302	40.6±1.5	588
3.60	15	10.2	16.7±1.0	255	30.2±1.3	412

[1] The number of animals required in each group for maximum accuracy is difficult to state. As in all biological assays, decreasing numbers of test animals yield results of decreasing significance. However, a statistical analysis of the data of this table indicates that the results obtained from random samples of 5 mice each are, in a large majority of cases, as accurate as those obtained from the individual groups of 15 mice each.

[2] The mean deviation of the mean (ϵ_M) was calculated according to Scott (10). This method of calculation sets up more rigorous standards than do the usual methods.

according to the routine Aschheim-Zondek technique. In every case the ovarian and uterine weights were greater in those animals autopsied after the 72-hour period. In other words, both the ovarian and the uterine weight actually decreased during the added 24-hour interval. Representative data of such experiments are given in table 1. As may be seen from these data, the greater response at the 72-hour point was obtained at all dose levels tested. The ovarian response was about 75 per cent greater and the uterine response 67 per cent greater than in the animals killed 24 hours later (96 hours after the first injection).

These results correlate well with those of Fevold and Hisaw (4) who injected immature rats with their hypophyseal follicle-stimulator and found a similar relationship between time and ovarian response. Evans et al (5) likewise reported a rapid response of the immature rat ovary to their hypophyseal synergist, which, according to later reports concerning its action in hypophy-

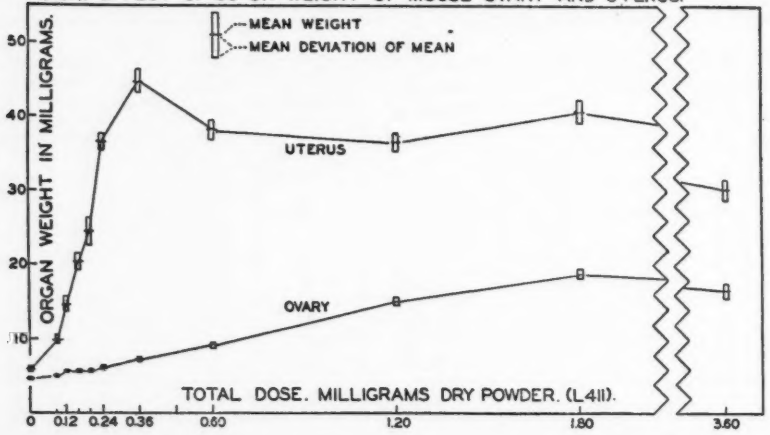
[4] CU = the gonadotropic material of castrate or post-menopausal urine.

sectomized male and female rats, appears to be very much like CU in nature (1, 6, 7, 8).

We have attempted to shorten the assay period even further by giving the entire dose of CU in one injection and autopsying the mice 30 hours later. The responses, however, were not as great as those obtained by either of the above described methods. Therefore, we have adopted as a routine procedure the division of the total dose into 3 equal injections given at 24-hour intervals[5] with autopsy 72 hours after the first injection.

Follicular Enlargement. Our numerous attempts to use follicular enlargement, as judged under the high power dissecting microscope, as a basis for quantitative assay have not proven successful. Not only is there a large sub-

FIG. 1. EFFECT OF CU ON WEIGHT OF MOUSE OVARY AND UTERUS.



jective factor in judging whether or not follicular size has been increased as compared to uninjected controls, but also, since this enlargement is very gradual with added increments of gonadotropin, an accurate quantifaction is practically impossible. Even if each ovary is sectioned and microscopically examined (which would be extremely laborious as a routine method) the question immediately arises as to just what degree of follicular enlargement should be used as the end-point. For quantitative work, if a definite end-point of this nature could be found, planimeter measurements, a time-consuming procedure, would be an absolute necessity. For these reasons we abandoned our attempts to use this criterion.

Ovarian and Uterine Reactivity. We have not been able to obtain entirely satisfactory assays of CU on the basis of increase in ovarian weight, a widely used test. In the rat, the extreme variability of the ovarian weight response, even among litter-mates, makes employment of this criterion almost impossible unless great numbers of animals are used (unpublished data). The weight response of the mouse ovary is less variable. However, in this form the rela-

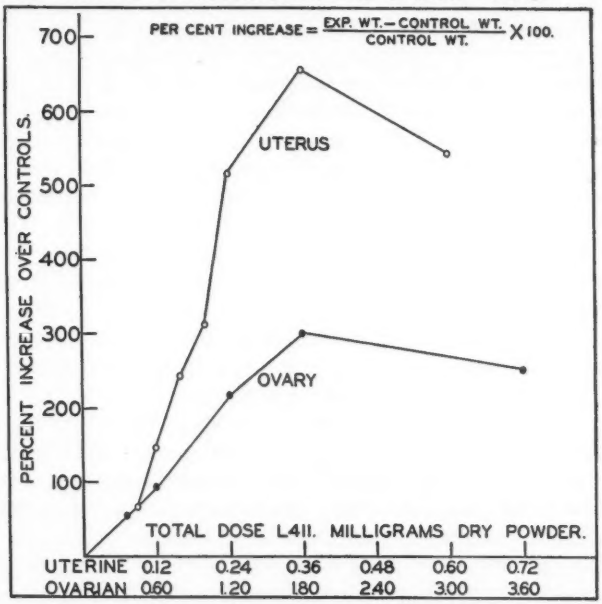
[5] Division of the total dose into more injections given at more frequent intervals would probably be better in a physiological sense. However, we feel that the additional labor involved is not justified by the slightly better responses which might result.

tively small weight change, anywhere within the reactive range, caused by altering the amount of administered gonadotropin does not permit quantitative determination of CU potency with error of less than 100 per cent (vide infra).

In only isolated cases have uterine weights been reported in assay work.[6] The following experiments were set up to compare the sensitivity of the ovarian and the uterine weights to small changes in administered dosage of CU. The results indicate the feasibility of using the mouse uterine weight as a basis for the assay of follicle-stimulating substances.

Graded doses of CU were administered to groups of immature mice. The

FIG. 2. RESPONSE OF MOUSE OVARY AND UTERUS TO C U.



highest dose in each series was such that further increase in dosage would produce no further increase in either ovarian or uterine weight. In all cases the total dose of gonadotropic material was administered in 3 equal injections, given at 24-hour intervals, and the animals were autopsied 72 hours after the first injection as described in an earlier section. A large number of preparations has been tested in this manner with very uniform results. Representative data obtained with one of these preparations (L411) is given in table 2 and figure 1. An inspection of these data shows that the ovary is functionally stimulated to produce estrin (as judged by uterine weight increase) by much smaller doses of CU than are necessary to produce comparable increases in ovarian weight. Any uterine weight increase is produced by but one-fifth to one-tenth

[6] Cahen and Ardoint have recently published a preliminary note (Compt. rend. Soc. de biol. 123:547, 1936) concerning the assay of pituitary gonadotropic material on the basis of the weight of the fixed and dried uteri of rats after 7-day treatment with the active material. This method appears to be more laborious and less accurate than that which we have been using during the past 3 years and which we report in this paper.

of the total dose required to produce an ovarian enlargement of the same magnitude.

Probably of even greater importance, as far as quantitative assay is concerned, is the fact that the uterine weight curve reflects small increases or decreases in total dose much more clearly than does ovarian weight, thereby permitting a much more accurate determination of CU potency. The data (table 2, fig. 1) indicate that in order to obtain significant differences in ovarian weight, it is necessary to make large changes in dosage. Even when an effective dose is halved or doubled, the change in ovarian weight is rather slight and may be obscured by individual variation unless large groups of animals are used. It is, therefore, apparent that quantitative assay of CU on the basis of ovarian weight response is subject to an error of at least 100 per cent.

On the other hand, if the dose is altered by as little as 25 to 30 per cent, anywhere within the range of uterine reactivity, distinct, easily measurable changes in the weight of this organ are obtained. In the case of preparation L411, total doses of 0.09, 0.12, 0.16, 0.20, 0.24, and 0.36 mg., produced uteri averaging 9.9, 14.6, 20.2, 24.3, 36.4, and 44.7 mg., respectively, representing increases over the control weight of 68, 147, 242, 312, 517 and 658 per cent. Differences of such magnitude are easily determined and readily reproduced and consequently permit assay with an accuracy of at least 25 to 30 per cent.

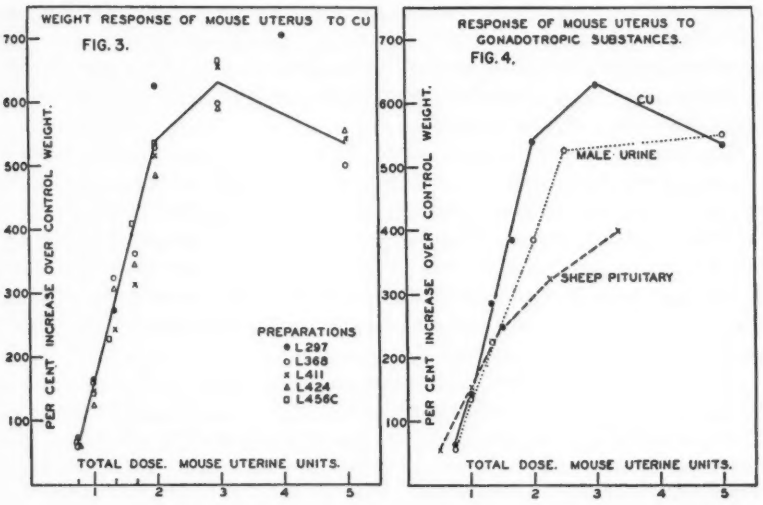
A direct comparison of ovarian and uterine sensitivity is given in figure 2. The percentage increase in weight of each organ, throughout its entire reactive range, is plotted against the total dose required to produce such change. The slope of each curve is a measure of the sensitivity of that organ to change in dosage. As is obvious from this comparison, the absolute and relative change in weight per each increment in dose is much greater in the case of the uterus than in the case of the ovary, clearly demonstrating that the uterine weight response is by far the more accurate indicator of the two.

The fact that the uterine weight response shows a linear relationship to dosage would, at first glance, lead one to believe that assay by interpolation from a standard curve would be feasible. However, as indicated in figure 1 and table 2, as dosage is increased the uterine weight reaches its maximum value rapidly and then, if still higher doses are administered, the weight curve falls off to somewhat lower levels. Since we have observed this repeatedly in both rats and mice treated with CU we believe that it is not due to an artifact. The decrease in uterine response when doses above the optimum are reached seems to make its appearance coincidently with the appearance of early luteinization. This correlation is especially marked in the case of the rat which seems more prone to luteinization than does the immature mouse. For this reason we have not attempted any final assays by interpolation from a standard weight curve.

We have arbitrarily defined the mouse uterus unit (so termed in order to avoid confusion with existing 'units') as that amount of CU which, when administered to each of 5 or more 21 to 23 day old mice in 3 equal portions

at 24-hour intervals produces on the fourth day (72 hours after the first injection) a 100 to 150 per cent increase in the average uterine weight. Such a unit takes advantage of the rapid change in uterine weight with change in dosage, is large enough to definitely distinguish the uterine weight from the normal weight and is small enough to avoid confusion with responses elicited by doses above the maximal. At this dose level, causing a doubling of the uterine weight and approximately coinciding with that required to cause vaginal canalization, the response is somewhat more constant than at higher doses.

The constancy of uterine response with different preparations is indicated by the data of figure 3. These data, obtained with 5 CU preparations of vary-



ing degree of purity, prepared and assayed at widely varying intervals, show that proportional increases or decreases in total dosage result in identical rise or fall in the uterine weight curves.

An obvious objection to the uterine reaction as a test for gonadotropic material is that estrogenic substance, if present, would render the results inaccurate. In assurance that this point need cause no difficulty, the following experience may be cited. A large number of our CU preparations, precipitated from the urine by tannic acid and washed with alcohol and acetone according to our published procedure (2) have been tested in several ways for contaminating estrogenic substance. In no case have we ever obtained any indication of the presence of estrogenic potency. Similar preparations made from human pregnancy urine and from normal male urine have likewise yielded negative results. Katzman and Doisy (9), using a similar method of preparation, have also found little, if any, estrogenic potency in their pregnancy urine extracts. It is, therefore, quite certain that if reasonable care is taken to remove

estrin which may originally be present there is little likelihood of error from this source.

The Response of the Uterus to Other Gonadotropic Substances. The successful results obtained in the quantification of CU potency led us to apply the uterine weight method to other types of gonadotropic material. Preliminary assays of gonadotropic extracts of normal male urine, sheep anterior pituitary, pregnant mare serum and human pregnancy urine were accordingly carried out using the same technique as described above for CU. The urinary preparations were prepared by tannic acid precipitation and subsequent purification as described for CU. The sheep pituitary extract was prepared according to the method of Fevold.[7] The pregnant mare serum preparation used was a commercial product.[8]

The results obtained with the gonadotropic material of normal male urine and with the pituitary extract are represented graphically in figure 4. The curves obtained with these substances are compared to the curve obtained with CU (taken from fig. 3). It may be seen that the male urine curve is essentially identical with that obtained with CU.

The uterine weight curve obtained with sheep pituitary extract differs somewhat from those obtained with the menopausal and normal urines. With the pituitary extract the curve plateaus much earlier, possibly due to the fact that this extract contained a much greater proportion of luteinizing material than did the urinary products. However, the first and important portion of the pituitary curve is identical with those of the CU and male urine extracts, indicating that the follicle-stimulating potency of this material may be readily assayed by means of uterine weight response.

Preliminary experiments with pregnant mare serum and with human pregnancy urine indicate that this method is not as accurate for these substances as when applied to CU. In the case of each of these chorionic substances the uterine weight elicited by minimal doses appears to be subject to considerable variation. With higher doses, producing uterine weights approaching the maximal, the response is more uniform. The reason for this lack of uniformity is unknown to us at the present time, although variable participation of the test animal's own pituitary may be involved in the explanation.

DISCUSSION

In the prepubertal female rodent the principle action of the gonadotropic material from the urine of non-pregnant individuals is the stimulation of the ovarian follicles. It follows that the best and most direct assay method for such substances should be based on an analysis of either increased function or size of the follicles. Of these two possibilities, the production of increase in function seems the more logical in a physiological sense. Moreover, change in follicular size is extremely difficult to determine with accuracy unless it be

[7] Private communication. According to Dr. Fevold this method completely or almost completely separates the follicle-stimulating and luteinizing substances. In the experiments reported here the FSH fraction was used.

[8] Antex Leo obtained through the courtesy of Dr. Christian Hamburger.

assumed that increase in ovarian weight is due entirely to follicular enlargement. Even if such an assumption is made and change in ovarian weight is used as a basis for quantitative assay, errors of 100 per cent or more cannot be avoided due to the relatively slight influence of change in dosage on the weight of the mouse ovary. The weight of the rat ovary is an even less accurate indicator of CU potency due to the great individual variation of response (unpublished data).

The hastened development of the follicles to the functionally active state causes the precocious secretion of relatively large amounts of estrin which in turn brings about several pronounced and easily measured changes, namely, precocious vaginal canalization, cornification of the vaginal epithelium and hypertrophy of the uterus. At first thought, precocious vaginal canalization appears to be the simplest of these three criteria of induced follicular secretion. However, this reaction does not, in our experience, lend itself well to our purpose, for with borderline doses of CU all gradations of response, the flat negative, the swollen but closed and the completely patent vaginal orifice are seen. Therefore, small increases or decreases in dosage cannot be determined accurately. Vaginal cornification can be ascertained with a certain degree of accuracy but the attainment of full cornification is a slower and less definite change than is the slight uterine hypertrophy required for assay purposes. Furthermore, by means of the uterine weight method a considerable degree of interpolation may be conveniently used for preliminary assay while the vaginal changes do not permit interpolation. The increase in uterine weight, therefore, appears to be the most adequate of these criteria for the measurement of induced follicular function.

The use of uterine weight as a basis for quantitative assay necessitates a rather small 'unit'. Such a unit is approximately equal to that necessary to produce vaginal canalization and is only about one-fifth the size of the usual 'ovarian-weight unit'. We believe that the small unit is advantageous especially in determination of urinary gonadotropic excretion of normal men and women.[9] In such determinations, involving small amounts of activity, it is exceedingly difficult to concentrate and detoxify the urinary gonadotropin (without loss of activity), sufficiently to permit the administration of enough material to produce a definite ovarian weight increase and yet not harm the test animal. It is, therefore, apparent that the uterine reaction, produced by smaller amounts of material and yet permitting much greater accuracy, is decidedly advantageous.

Although the degree of accuracy with which follicle-stimulating substances may be assayed by the uterine weight method is much greater than that of any other method that has been brought to our attention, it still leaves much to be desired. The usual sources of error attendant upon any method of biological assay are here present. However, we are of the opinion that, in the light of present knowledge, the uterine weight method offers the best available com-

[9] Our preliminary data indicate that by means of tannic acid concentration and uterine weight assay, the gonadotropic potency of a single 24-hour specimen of normal male or female urine may be accurately and easily determined.

bination of accuracy, simplicity and ease for the quantitative assay of so-called 'follicle-stimulating' substances.

SUMMARY

Assay of follicle-stimulating potency on the basis of the weight response of the mouse ovary involves a possible error of at least 100 per cent.

The weight of the mouse uterus is relatively and absolutely a very sensitive indicator of follicle-stimulating potency and affords a simple, accurate criterion for quantative assay. Assay of follicle-stimulating substances (castrate or post-menopausal urine) on the basis of mouse uterine weight is at least 3 times as accurate as that based on the ovarian weight. Preliminary experiments indicate that quantitative assay of the follicle-stimulating activity of normal male and female urine and of sheep pituitary may also be conducted on the basis of the mouse uterine weight. The "mouse uterus unit" has been defined as the smallest amount of material which, when administered to each of 5 or more 21 to 23 day old mice in 3 equal portions at 24-hour intervals, produces on the fourth day (72 hours after the first injection) 100 to 150 per cent increase in the average uterine weight.

REFERENCES

1. Leonard, S. L., and P. E. Smith: Am. J. Physiol. **108:** 22. 1934.
2. Levin, L., and H. H. Tyndale: Proc. Soc. Exper. Biol. & Med. **34:** 516. 1936.
3. Aschheim, S., and B. Zondek: Klin. Wchnschr. **7:** 1404. 1928.
4. Fevold, H. L., and F. L. Hisaw: Am. J. Physiol. **109:** 655. 1934.
5. Evans, H. M., M. E. Simpson and P. R. Austin: J. Exper. Med. **58:** 545. 1933.
6. Smith, P. E., E. T. Engle and H. H. Tyndale: Proc. Soc. Exper. Biol. & Med. **31:** 745. 1934.
7. Evans, H. M., R. I. Pencharz and M. E. Simpson: Endocrinology **18:** 601. 1934.
8. Evans, H. M., R. I. Pencharz and M. E. Simpson: Endocrinology **18:** 607. 1934.
9. Katzman, P. A., and E. A. Doisy: J. Biol. Chem. **98:** 739. 1932.
10. Scott, E. L.: J. Biol. Chem. **73:** 81. 1927.

EPITHELIAL GROWTH CAUSED BY STIMULATION WITH VARIOUS SMEAR METHODS AS DEMONSTRATED BY MITOTIC STASIS WITH COLCHICINE

PHILIP V. ROGERS AND EDGAR ALLEN
From the Department of Anatomy, Yale University School of Medicine
NEW HAVEN, CONNECTICUT

It has been reported by Wade and Doisy (1) that vaginal smears repeated 2 or 3 times a day cause an increase in the number of epithelial layers of the vagina of the spayed rat and sometimes confuse the result of tests of estrogens. Twenty-five per cent of the animals smeared 3 times a day showed cornification at the end of the third day. Smears were made with swabs, "a small amount of cotton (wrapped) about the end of a toothpick," the swab being rotated a few times in the vagina. It seemed to us that this might explain some of the variable results reported by Young (2) after frequent smearing in the guinea pig during heat.

The following experiment was carried out in order to determine whether frequent stimulation of the vagina by the cotton swab method of making smears would induce growth of the epithelium to a degree which would confuse the usual sequence of cells in the smear. For controls, other animals were smeared gently by inserting uncovered toothpicks. In these experiments we had the added advantage of the use of colchicine, which makes it possible to accumulate additional evidence of growth of the vaginal epithelium under the two methods, for a single injection of colchicine arrests in metaphase all cells dividing during a period of 8 to 10 hours (3). By this method Allen, Smith and Gardner (4, 5, 6, 7) have demonstrated growth of vagina and mammary glands after theelin administration, proliferation of the follicle cells, theca and germinal epithelium of the ovary, and growth of the endometrium and myometrium. Reynolds (8) has shown that distention of the rabbit's uterus by insertion of paraffin pellets is sufficient stimulation to increase the size of the uterus. Allen, E., Smith and Reynolds (9) have demonstrated by the colchicine technique that rapid hyperplasia of uterine muscle on the third day of distention contributes to this effect.

EXPERIMENTAL RESULTS

Fifteen normal adult rats were spayed at least 1 week before the beginning of the experiment. Daily vaginal smears (without trauma) were taken throughout this time to make certain that the vagina was relatively quiescent. Rats castrated for longer periods were 'primed' with theelin 1 week before experimentation was started in order to have the condition of the vagina as nearly as possible the same in all animals. The controls consisted of, *1)* unsmeared rats

and, *2)* castrate animals brought into full vaginal cornification with injections of theelin. The controls also received colchicine 8 hours before autopsy.

Two types of vaginal smears were made. In the first method a moist toothpick was inserted into the lumen of the vagina, turned gently a half revolution, and withdrawn. An adequate number of cells for reading the smear was always obtained. In the second method a fair sized piece of cotton was wrapped about the end of the toothpick. The cotton covered swab was then rotated several times in the vagina during the process of taking the smear. Eight hours before autopsy each animal received a subcutaneous injection of 0.2 mg. of colchicine.

At autopsy the entire reproductive tract was removed and fixed. The vagina

TABLE 1.

Rat	Smear technique	Autopsied (Hr. after 1st smear)	'Colchicine' mitoses in vaginal epithelium	Number of epithelial layers
Controls	—	—	—	2– 3
Experimental:				
1	moist toothpick (3×)	32	±	2– 3
2	moist toothpick (3×)	32	±	2– 3
3	moist toothpick (3×)	32	+	2– 4
4	moist toothpick (3×)	32	±	3– 6
5	cotton swab (3×)	32	++++	6–10
6	cotton swab (3×)	32	+++	3– 6
7	cotton swab (3×)	32	++++	4– 6
8	cotton swab (3×)	32	+++	6– 9
12	cotton swab (3×)	48	+	2– 6
13	cotton swab (3×)	48	+	4– 6
14	cotton swab (3×)	57	++	6–10
15	cotton swab (3×)	57	+	3– 6
9	cotton swab (7×)	80	+	6–10
10	cotton swab (7×)	80	++	2–10
11	cotton swab (7×)	80	+++	10–12

was then cut in half and sections made so that the region studied was in each case about half way between the cervix and the external orifice.

Rats Killed at the End of 32 Hours

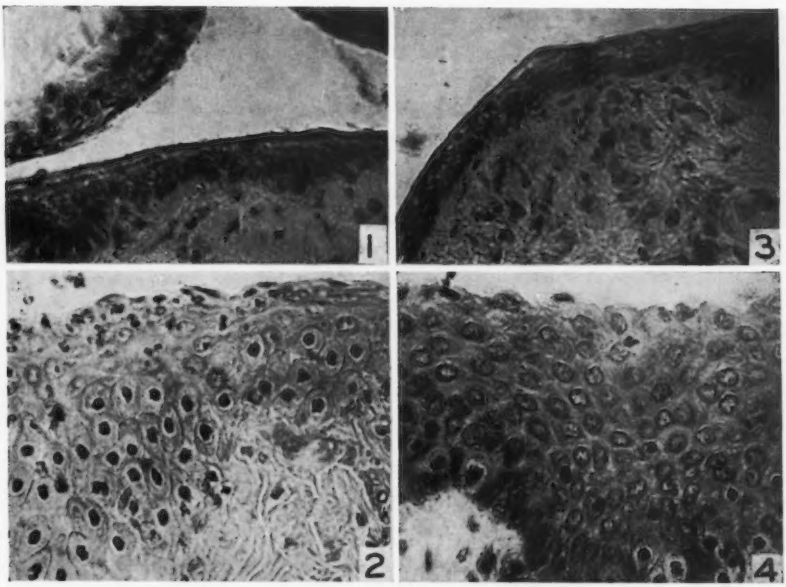
Smeared gently with moist toothpicks. Vaginal smears carefully made with a moist toothpick were taken on 4 spayed rats at the start of the experiment, at the 8th and at the 24th hour afterwards. Colchicine (0.2 mg.) was injected into each animal when the last smear was made and the animal was killed 8 hours later. Sections through the vaginae (fig. 1) showed a typical castrate type of epithelial wall of from 2 to 4 layers (table 1). The colchicine effect was similar to or only slightly more marked than in the control castrate animals receiving the drug but not smeared (fig. 3). The vagina of 1 of the 4 experimental animals contained a fair number of mitotic figures and is classed as 1+ in the table. This is compared with a plus minus for the other 3 rats and minus for the controls.

Smeared with cotton swab. Four rats received exactly the same treatment as those above except that a cotton swab was used to take the smears instead of a plain toothpick. Sections of the vaginae showed a marked accumulation of

epithelial mitoses arrested by colchicine,—3 and 4 plus (fig. 2). The thickness of the epithelium had likewise increased.

Rats Killed at the End of 48 and 57 Hours

Four animals were smeared with a cotton swab on the 1st, 8th, and 24th hour as in the above series. Administration of colchicine, however, was delayed until the 37th hour. Two of the 4 were autopsied 11 hours later. The remaining 2 received a second dose of colchicine at this time and were killed at the 57th hour. The results of both groups were similar so will be considered



Sections of vaginal epithelium of ovariectomized rats; mitoses arrested by administration of colchicine. x 390.

1. Thirty-two hours after first vaginal smears made with a moist toothpick (note absence of mitoses).
2. Thirty-two hours after first of 3 vaginal smears made with cotton swabs. A considerable thickening of the epithelium and many mitoses arrested in metaphase by colchicine. These represent cells dividing during the last 8 hours of the 32 hour period.
3. Control castrate, not smeared, showing absence of cell division.
4. Partial growth reaction eighty hours after first of 7 smears made during 3 days with a cotton swab. No cornification is present; the smears remained negative.

together. The proliferation of vaginal epithelium was much less marked (1+ and 2+) than in those autopsied on the 32nd hour, but greater than in the controls and in those animals smeared gently with moist toothpicks. The number of cell layers of the epithelium was about the same as in those rats killed after 32 hours (table 1). The superficial epithelial cells were not cornified but seemed somewhat similar to those taking part in a mucification reaction (10).

Rats Killed at the End of 80 Hours

Three animals were smeared with a cotton swab at the following hours: 1, 8, 24, 32, 48, 56, and 72. At the 72nd hour, colchicine was administered. All 3 rats were killed 8 hours later. The rate of epithelial proliferation was

greater than in those animals receiving only 3 vaginal stimulations and killed after 48 hours, but less than in those autopsied after 32 hours (table 1). Although the rate of cell proliferation was less than in some rats, the number of layers of the vaginal epithelium had increased more than in any of the other animals. The epithelium of 1 rat (fig. 4) was 10 to 12 cell layers thick. There was, however, no sign of cornification.

DISCUSSION

The work reported in this paper is one more demonstration of the value of using colchicine when studying growth of tissue. It makes it unnecessary to carry an animal for 60 or 70 hours to see if there is an increase in the number of layers of the vaginal epithelium when a mitotic count can be made at 30 hours or earlier. Although we were unable to demonstrate any cornification of the vagina at 48, 57 and 80 hours after the initial stimulation, the decided epithelial growth found in those rats smeared with a cotton swab makes it advisable to use great care in the method of obtaining the smear. A sufficient quantity of cells may be obtained from the vaginal lumen with a moist toothpick (as shown in this paper) without causing the vaginal growth induced by a cotton swab.

SUMMARY

Using the colchicine technique, a comparative study of the degree of epithelial stimulation resulting from the taking of vaginal smears was made on 15 experimental rats. The controls consisted of a number of castrate animals receiving only colchicine and a series of rats injected with colchicine after being brought into estrus with theelin. Rats carefully smeared with a moist toothpick showed very little cell proliferation when compared with those smeared with a cotton swab. The greatest stimulation was present about 32 hours after first using the cotton swab. At the end of 80 hours there was an increase in the thickness of the epithelium but no sign of cornification.

A cotton swab rotated several times in the vagina caused a stimulation of the epithelium which manifested itself by an increase in dividing cells and thickness of epithelium. Vaginal smears made with a moist toothpick, on the other hand, failed to bring about these changes.

REFERENCES

1. Wade, N. J., and E. A. Doisy: Proc. Soc. Exper. Biol. & Med. **32:** 707. 1935.
2. Young, W.: Anat. Rec. **67:** 305. 1937.
3. Dustin, A. P.: J. des Sci. Med. de Lille. No. **49:** 561. 1934.
4. Allen, E., G. M. Smith, and W. U. Gardner: Anat. Rec. **67:** 49. (Suppl. 1) 1936.
5. Allen, E., G. M. Smith, and W. U. Gardner: Anat. Rec. **67:** 3. (Suppl. 3) 1937.
6. Allen, E., G. M. Smith, and W. U. Gardner: Endocrinology **21:** 412. 1937.
7. Allen, E., G. M. Smith, and W. U. Gardner: Am. J. Anat. **61:** 321. 1937.
8. Reynolds, S. R. M., and S. Kaminester: Am. J. Physiol. **116:** 510. 1936.
9. Allen, E., G. M. Smith, and S. R. M. Reynolds: Proc. Exper. Biol. & Med. 1937 (In Press).
10. Meyer, R. K., and W. M. Allen: Anat. Rec. **51:** 21. (Suppl. 1) 1931.

OVARIES SECRETE MALE HORMONE
III. Temperature Control of Male Hormone Output by Grafted Ovaries[1]

R. T. HILL
From the Department of Anatomy, Yale University School of Medicine
NEW HAVEN, CONNECTICUT

A normal man excretes appreciable amounts of female hormone (1), and women excrete variable amounts of male hormone (2). From where does the secretion of a specific sex hormone come in an individual of the opposite sex? Both the testis and the ovary have been held responsible for a dual secretion and physiology, thereby accounting for two opposite sex hormones being elaborated in an unisexual organism. It is well known that in some lower forms maleness and femaleness may exist in the same individual, and a type of sliding scale may be applied to the ratio of the sex determiners present (3). It is possible in these forms to have an individual act as male in some matings and as female in others, depending upon the degree of maleness or femaleness in the organism to which it is mated.

Avian physiology and anatomy furnish some remarkable cases of dual sex function in a single individual, either in sequence or simultaneously. However, most, if not all, of these cases can be explained by the anatomy of the existing gonads (see 3, for references and complete discussion).

In three previous reports (4, 5, 6) evidence was presented which proved that in mice female gonads can be induced to assume the endocrine function of testes. When the discovery was first made that grafted ovaries could activate the sex accessories of castrated male mice, it was noted that only those gonads which were grafted in the ear produced the response. Ovaries grafted in the region of the abdomen did not act in the same way. As an explanation the probability was mentioned that the ear was normally cooler than the abdomen, and that the lower temperature to which a gonad was subjected when grafted in the ear might enter into the reaction obtained. It also seemed probable that, since the ear of a mouse extends a considerable distance from the body and is a paper-thin structure, its temperature, and likewise the temperature of any graft existing therein, could to some degree be controlled under different surrounding thermal changes. Working on this basis, the following experiments have been performed, and evidence is presented here which shows that temperature can play a part in the physiology of a grafted ovary.

EXPERIMENTAL

A group of 12 male mice was castrated, and at the same time an ovary from a sister was grafted under the skin of both ears of each animal. Six of

[1] This investigation was made possible by a grant from the Fluid Research Funds of Yale University School of Medicine.
All mice used were from L. C. Strong's inbred colony.

the newly grafted males were put in a chamber with a constant temperature of 22°C. The remaining 6 animals were placed in a second chamber at 33°C. The animals were so assorted that for each mouse at one temperature (22°C.) a full brother was put at the other maintained temperature (33°C). All animals used in this experiment were of similar ages (sub-adult), and all the operations were performed on the same day, the only variable introduced into the experiment being that of temperature. At various intervals throughout the experiment laparotomies were performed on all 12 animals to ascertain the general condition of the seminal vesicles and prostates. In some animals a series of biopsies were taken in order to follow the histological picture.

The first important change noticed was that at 55 days after grafting, 3 of the castrated males in the cool environment were found to have fully de-

TABLE 1. CASTRATE MALES WITH OVARIES GRAFTED IN EARS

Animal No.	Surrounding Temp. °C.	Seminal vesicle and prostate response Days after grafting 55	83–85	107	156
M.S V. 1	33	−	−	−	−
" 2	33	−	−	−	−
" 3	33	−	−	−	−
" 7	33	−	−	−	−
" 8	33	−	−	−	−
" 9	33	−	−	−	−
M.S.V. 4	22	−	−	+	+
" 5	22	−	+	+	+
" 6	22	−	−	−	+
" 10	22	+[1]	−[2]	+[3]	
" 11	22	+[3]			
" 12	22	+	+[4]	−[5]	+

[1] Put at 33°C from 55-85 days after grafting.
[2] Put at 22°C from 85-107 days after grafting.
[3] Killed.
[4] Put at 33°C from 85-107 days after grafting.
[5] Put at 22°C from 107-156 days after grafting.

veloped seminal vesicles and prostates (table 1). Thus, by controlling the temperature, the time necessary for ovarian tissue to assume a physiological male hormone output was reduced by about one-half of that previously reported (Hill, 1937a). The remaining 3 individuals in the cool chamber required 85, 107 and 156 days respectively for the same reaction to take place. This fact illustrates the part that individual variation plays in these experiments.

In striking contrast, all laparotomies on the 6 animals which were maintained at 33°C. over a period of 156 days showed that the seminal vesicles and prostates had not been activated, being completely castrate in type. Thus it may be seen that those animals kept in a cool environment (22°C.), where the temperature of the grafted ovary approximated that of the normal scrotum, registered a 100 per cent positive response in activation of the sex accessories. Also, when the surrounding temperature was kept near that of the body (33°C.), the activation of the seminal vesicles and prostates by the grafted ovary was 100 per cent negative. These results are clear cut and conclusive and their significance cannot be overlooked.

Two animals, 10 and 12, table 1, offer additional evidence to support the idea that temperature affects the reaction obtained. The first animal, 10, had developed normal accessories under the influence of grafted ovaries and a temperature of 22°C. at 55 days after grafting (table 1 and fig. 1, *A*). It was then put in a cage at 33°C. for 30 days, during which time the seminal vesicles and prostates underwent complete castrate atrophy (fig. 1, *B*). This animal was then returned to its former cage (22°C.), and at the end of 22 days the accessories were again found to be normal (fig. 1, *C*). The same procedure was followed for the second animal, 12, but at a different time, and its responses were found to be identical.

Of further interest is the fact that in one animal, 5, the seminal vesicles and prostates have been maintained for more than 100 consecutive days. This

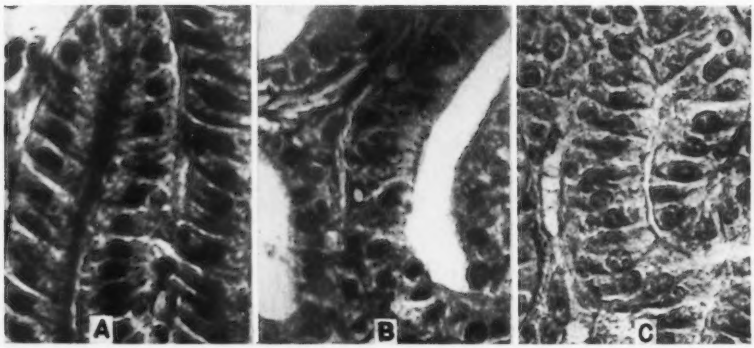


Fig. 1. Seminal vesicle epithelium of M.S.V. 10, a castrate male. *A*), Brought to full activity by ovaries grafted in ears, and an environmental temperature of 22°C for 55 days after grafting. x 610.
B), Showing complete castrate atrophy as a result of an environmental temperature of 33°C for 30 days (55-85 days after grafting). x 610.
C), After a second period of activation in a surrounding temperature of 22°C for 22 days (85-107 days after grafting). x 610.

shows that there is no detectable rhythmicity in the male hormone output of a grafted ovary when a temperature of 22°C. is maintained.

DISCUSSION

It is not yet known in just what way temperature affects the physiology of a grafted ovary. As previously pointed out (4, 5) the histology does not significantly change with changes in temperature, at least in instances of short duration. The effect of environmental temperature over extended periods remains unknown for the present. In birds with abdominal gonads in both sexes, and in which both the ovaries and the testes are under like thermal conditions, temperature cannot be an important factor in gonadal physiology. It therefore seems impossible at present to draw any correlation between sex reversal and physiology in avian gonads and the work here described. The same seems to be true of amphibian gonadal development and function.

It is not considered, in these experiments, that any type of sex reversal is dealt with. Instead it would seem that the technique used has only served to manifest a function which probably exists, to a small degree, in the gonads of most normal females. The relatively large number of researches on the excre-

tion of male hormone in the urine of women substantiates such a statement.

In the male with experimental cryptorchid gonads, the endocrine function disappears very slowly and over a period of time up to a year or more (7). In the light of this fact, it is hard to understand how a grafted ovary can lose its physiological output of male hormone in 3 to 4 weeks when the host animal is moved from cool to warm surroundings. The suggestion is made that perhaps the grafted female gonads elaborate amounts of male hormone just sufficient to reach the threshold necessary to activate the castrate male accessories, and that an increase of temperature quickly reduces the amount below that threshold. Chemically, male and female hormones are quite similar. Does temperature enter into the metabolism, within an ovary, to change one sex hormone to or from the other, or are both sex hormones elaborated as separate entities? Does general body metabolism of the organism affect the response obtained from grafted ovaries? As yet it has not been demonstrated that grafted testes can elaborate physiological amounts of female hormone. Is the mammalian female gonad then the 'dominant' type and capable of elaborating two sex hormones? These and many other questions can only be answered by future experiments. Certainly, the effect of temperature in these experiments does not appear to be directly upon the hormone after its formation or on its availability to the organism, but rather upon that tissue which gives rise to the hormone.

SUMMARY

Ovaries grafted in the ears of castrated male mice secrete amounts of male hormone sufficient to maintain normal seminal vesicles and prostates. The male hormone secretion of grafted ovaries can be considerably enhanced by continually subjecting the gonad to cool thermal surroundings (22°C.). Male hormone secretion is not detected, if the ovary grafted in the ear is maintained at near body temperature (33°C.), for at least 156 days.

A cycle of male hormone activity and quiescence can be created by changing the temperature of the grafted gonad at 3 to 4 week intervals. Temperature changes of short duration have not been noticed to affect the histology of the graft.

Grafted ovaries can maintain the accessories of a castrated male mouse for at least 100 consecutive days.

REFERENCES

1. Dorfman, R. I., T. F. Gallagher and F. C. Koch: Endocrinology **19:** 33. 1935.
2. Koch, F. C.: Physiol. Rev. **17:** 153. 1937.
3. Allen, E.: Sex and Internal Secretions. Chap. V. Williams and Wilkins, Baltimore, 1932.
4. Hill, R. T.: Endocrinology **21:** 495. 1937.
5. Hill, R. T.: Anat. Rec. **67:** (Suppl.). 24. 1937.
6. Hill, R. T., and W. U. Gardner: Anat. Rec. **64:** (Suppl.). 21. 1936.
7. Nelson, W. O.: Proc. Soc. Exper. Biol. & Med. **31:** 1192. 1934.

ACCELERATION OF THE RATE OF PASSAGE OF FERTILIZED OVA THROUGH THE FALLOPIAN TUBES OF MICE BY MASSIVE INJECTIONS OF AN ESTROGENIC SUBSTANCE[1]

H. O. BURDICK AND RAE WHITNEY
From the Department of Biology, Alfred University
ALFRED, NEW YORK

In earlier experiments on the tube-locking of fertilized ova by small doses (7.5 to 25 R.U.) of estrogenic substances, it was noticed that in a few animals some of the ova were located in the upper end of the fallopian tube (1). Under normal conditions the ova would have been in either the uterine third of the tube or in the uterine lumen, according to the lapse of time after fertilization. Since ova were occasionally found in the upper tubal region, particularly after injections of 25 R.U. of an estrogen, it was thought possible that increased doses resulted in a proövarial peristalsis. Experiments were undertaken to determine this possibility. However, we have found that increased injections of an estrogen result in neither a proövarial peristalsis nor the tube-locking of ova as with smaller amounts but, instead, result in an acceleration of the rate of passage of ova through the tubes.

Copulation in mice was determined by the vaginal plug method, and the estrogen used was progynon-B in oil. Autopsies were performed 11 to 64 hours after vaginal plugs were found. All injections and autopsies are listed in this paper in hours after the vaginal plug (a.v.p.). At the autopsy each uterine horn was carefully removed with its fallopian tube and the attached ovary. Each ovary was examined for corpora lutea of ovulation.

For examining the tracts and keeping ova for photographing, a 0.5 per cent saline solution was used. This concentration had been found best for mouse ova before the blastula stage (2). However, when ova had been in the uterus for some time they presented a fused appearance and frequently the number of blastomeres within a zona could be determined only by counting the denser nucleoli. Such eggs maintained this fused appearance if placed in 0.3 per cent saline, but became more or less normally spherical when kept in 0.5 per cent saline solution or placed for a few moments in Ringer's solution.

The uteri of animals autopsied less than 45 hours a.v.p. were frequently found to be filled with old seminal fluid. If corpora lutea of ovulation outnumbered the ova recovered in either tubes or uterus it was assumed that the ova might have descended into the uterus, where they would be obscured by

[1] These experiments were made possible by grants from the Penrose Fund of the American Philosophical Society and from the Division of Biology and Agriculture of the National Research Council. The funds were administered by Mr. Curtis F. Randolph, Treasurer, Alfred University.

the seminal fluid. Material on the fate of ova whose passage has been accelerated through the tubes will be presented in the near future.

In the tabulations in this report we have followed the method established by Lewis and Wright (3) in listing each egg by a separate number which also indicates the stage of that egg. Letters are used as follows: *D* (degenerate), *F* (fragmented), *N* (not fertilized), and *Z* (empty zona pellucida).

Mouse ova normally descend into the uterus 72 to 96 hours after copulation (4, 3), however, our earlier experiments with injections of estrogens had shown that the descent of ova into the uterus was prevented. Therefore after the first autopsies in these experiments showed a frequent discrepancy in the total of recovered ova compared with the number of corpora lutea, we reexamined the solutions in which the uterus and tube were first separated. Ova were frequently flushed from the uterus but they were more often found in the short portion of the uterus which had been cut off with the intact tube. After the discovery of eggs in this region of the uterus particular care was exercised in the removal of the tracts from the animals so that pressure on the tubo-uterine region was avoided.

Several times in these experiments ova were seen just at the uterine ostium of the tube or touching the lips of this opening. In other animals a slight pressure on that portion of the tube which protrudes into the uterine lumen forced the eggs into the saline solution. This condition was never encountered after injections of 7.5 to 25 R.U. of estrogens. If it is possible that a stretching of the uterus during the removal of a tract had sucked the ova from the tubes into the uterus, then the physiological and anatomical conditions at the end of the tube must have been radically different from these in experiments with tube-locked ova. In these latter experiments, the manner of handling the tract never resulted in descent of ova into the uterus.

In the first series, with injections of 100 R.U., at the time of the vaginal plug, ova were usually found in the tubes at autopsies less than 72 hours. In 3 cases all or several ova in each animal were in the uterus at 42, 49, and 51 hours, a.v.p. These were in the 2-celled stage. When injections of 100 R.U. were delayed 24 to 32 hr. a.v.p. in a series of 10 animals, the ova were still in the tubes at 31 to 53 hr. a.v.p. in 5 animals, but were in the uterus in 5 others at 40 to 52 hr.

In a series of 7 animals receiving 200 R.U. of estrogenic substance once within 25 to 32 hr. a.v.p., all were found to have ova either in the uterus or in the uterus and tubes when autopsied at 51 to 65 hr. In one of these, the ova were first seen within the ostium or uterine end of the fallopian tube, retained in the tubal fluid which seemed to be more viscous than the fluid of the uterine lumen. Other animals in which injections of the same amount were delayed to 48 hr. a.v.p. had ova in the uterus or uterus and tubes at 59 and 60 hr. a.v.p.

A third series of 7 animals was injected with 300 R.U. within 3 hours after the plug. Two of these autopsied 11 and 26 hr. later, had retained ova in the tubes. Five others autopsied 33 to 56 hr. a.v.p. had ova within the uterus or uterus and tubes. When injections were delayed 11 hr. a.v.p. in 5

animals, one showed only tubal ova at 36 hr. a.v.p. while 4 had ova in the uterus or uterus and tubes at 33, 36, 37 and 64 hr. A fourth series received injections of 500 R.U. At autopsy 37 to 56 hr. a.v.p. 2 were found with ova in the tubes at 37 and 56 hr. a.v.p. while 3 had ova within the uterus or uterus and tubes at 37, 38 and 51 hr. a.v.p.

That the injections were not consistent in accelerating the passage of all the ova in similar experiments of controlled time, dosage and hours of autopsy

Animal	Time Injected, hours a.v.p.	Autopsy, hours a.v.p.	Tube		Uterus	
			Left	Right	Left	Right
			One Injection, 100 R.U.			
121	0	52	6666	—	—	—
119	0	51	4	—	—	—
118	0	50	234	44	—	—
116	0	49	43	1	222	—
133	0	48	—	4	—	—
131	0	43	—	44	—	—
130	0	42	4	—	2211D	222D
88	22	53	8	6810	—	—
85	22	51	—	4	44446	44D
126	23	36	2222	1222222	—	—
127	23	37	2	22	—	—
128	23	37	22	22222	—	—
129	23	40	—	4444	44444	—
122	31	50	13	—	—	122
123	31	51	23445	—	—	3
124	31	52	233	44444	—	—
125	31	52	—	—	FFF	FFF
			One Injection, 200 R.U.			
95	0	52	N	8	—	—
32	16	51	—	—	4	N6
112A	25	54	—	288	44	8
112B	25	51	8	4	6	57
111	25	51	1112D	—	—	44
136	32	56	—	—	48888	8888Z
139	32	62	—	—	DD	DD8
138	32	64	—	DD	F	FD
137	32	64	DDD	—	88	8
63	48	59	—	—	2222	—
67	48	60	22	—	—	12
			One Injection, 300 R.U.			
57	0	33	—	—	22224	No record
72	3	11	1111	11111	—	—
76	3	26	222222	222	—	—
74	3	34	2	22	22223	—
75	3	36	—	112222	222	—
78	3	51	2	—	2	2
73	3	56	8	—	—	8
68	11	33	22	22	2	—
49	11	36	—	—	2222	22
70	11	36	2	22	—	—
69	11	37	—	223	2	—
45	11	64	—	—	one 16-celled	—
			One Injection, 500 R.U.			
101	0	37	—	—	—	222?
104	0	37	—	2	2222233	—
102	0	38	Z	—	224	2233 (tip)
103	0	51	N	—	F6 (fused)	—
105	0	56	66	666	—	—

Animal	Time Injected, hours a.v.p.		Autopsy, hours a.v.p.	Left Tube	Right Tube	Tract Uterus	Tract Uterus
	First	Second			*Two Injections, 100* R.U.		
141	0	6	29	—	—	D	2
140	0	6	27	—	6	66	66N
142	0	6	30	2222	22	—	—
143	0	6	31	222222	22	—	—
144	0	6	31	222	222	—	—
27	5	30	39	6	D	—	—
26	5	30	57	D8FF	—	—	10
89	10	25	53	446	—	22	4
90	10	25	54	—	—	4 (tip)	44
24	30	46	52	66	—	6	8888
25	30	46	56	6	—	4	D57
					Two Injections, 200 R.U.		
29	1	25	48	144 (fused)?	—	—	—
37	0	32	48	6	8	—	—
62	1	25	51	—	—	24	—
35	0	32	52	666	—	—	—
38	0	32	54	—	—	8	—
110	5	9	26	112222	222	—	—
108	29	31	36	2222	222	—	—
109	29	31	38	22	D2222	—	—
					Two Injections, 300 R.U.		
106	13	25	39	44	—	—	22
107	13	25	55	88	D8	8	DD8
105	13	25	56	66	666	—	—

a.v.p., was very puzzling. Thinking that the threshold for the elimination of estrogen from the blood might differ in the various animals, another series of mice was injected with a second dose of estrogen. It was thought possible that should the action of the estrogen be inhibited too soon for the completion of the tubal descent of ova then a second injection might bring this about.

In one small series injected with 100 R.U. of progynon-B at v.p. and again 6 hours later, 3 had only tubal ova at 30, 30½, and 31 hr. a.v.p., while 1 had ova in the uterus at 28½ hr. a.v.p. Another, at 27 hr., had ova in the uterus and tubes. Of 5 animals injected the first time 8 to 10 hr. a.v.p. and again 14 to 24 hr. later, 2 showed tubal ova 38 and 39 hr. a.v.p. while 3 were observed with uterine or uterine and tubal ova at 53, 54 and 57 hr. a.v.p. Another 2 animals received the first injection 24 hr. a.v.p. and the second injection 15 hr. later. At autopsy, 52 and 56 hr. a.v.p., in both cases ova were found only in the uterus.

Mice injected with 200 R.U. twice did not show much more uniform results than did those receiving 200 R.U. once. However, in 3 of this series (108, 109, 110), probably the autopsies were performed too soon for the estrogen to have taken effect. In the other 5 animals of this series too few ova were found either in the tubes or uterus. According to the number of corpora lutea there should have been an average number of ova in each mouse. Two of these showed ova in the uterus only.

The series of animals receiving 300 R.U. twice is small, but 2 of the 3 showed eggs in the uterus.

DISCUSSION

In looking over the tables of results, it is of interest to note that ova were frequently recovered from the uterus in the 2-celled stage. The earliest 2-celled ova were seen in the uterus 29 hr. after the vaginal plug (animal 141, injected with 100 R.U. twice), while others were obtained 33 to 36 hr. a.v.p. In general, the egg stages parallel those of Lewis and Wright, but we cannot account for 2-celled stages as late as 50 and 60 hr. a.v.p.

Of 67 animals injected, 41 or 61 per cent had ova in the uterus or uterus and tubes before the usual time of their descent. Of those recorded with only tubal ova several were probably autopsied too early for the estrogen to have become effective (126, 128, 13-14 hr. after injections). On the other hand, ova were still in the tubes in some animals (116, 118, 119, 121) 50 hours after injections.

A delay of about 30 hours after the plug for the first injection of 200 to 500 R.U. was apparently as effective as injections at the time of the vaginal plug. It is also evident that ova are not generally in the uterus until about 25 hr. after injection although in a few cases (122, 123) only approximately 20 hours had elapsed between injections and the finding of ova in the uterus.

Although these experiments have not yielded uniform results, ample proof is given to show that injections of 100 to 500 R.U. of progynon-B usually result in the acceleration of the rate of passage of ova through the tubes of mice. That this phenomenon may occur in normal animals is shown in Lewis and Wright's (3) table 6, animals 31 and 39, in which the eggs were found in the uterus in the 8-celled stage. This, however, is an exception and we have found no mention of other cases in the literature.

Wislocki and Snyder's experiment (5) does not show acceleration of the rate of transport of the fertilized ova. In their experiments, pregnancy urine extract was used to induce a second ovulation 4 days after the first eggs, thrown off as a result of copulation, had been fertilized. The total time that the first group of eggs was retained in the tubes was apparently normal, but the eggs of the second ovulation reached the uterus less than 59 hours after injection. It is only these latter ova which were 'accelerated.' It seems that this descent of the second cluster of ova with the first set should be expected. The forces at work moving the first eggs also moved the second. But what were the forces which caused or allowed the first group of eggs to descend into the uterus?

In seeking an answer to this question, our attention was directed to the developing corpora lutea. In these experiments, injections of massive doses of progynon-B resulted in an early increase in size of the corpora lutea. They were frequently as large as those of late pregnancy. However, a few tentative experiments with progesterone (proluton) have not resulted in an early descent of mouse ova. Furthermore the use of progesterone would not seem logical since several investigators (6, 7, 8, 9, 10, 11) have shown that this hormone causes quiescence of the uterus and that estrogenic substances induce rhythmical contractions of the rabbit uterus even in hypophysectomized animals.

One possible cause of variation may be in the method of injections. There

may be some back flow of the oily progynon-B as the animal fur will sometimes appear as if it had come in contact with oil in the cages. Furthermore, it is possible that the needle may be thrust into subcutaneous areas which differ in absorption capacities.

More consistent results were expected with injections repeated a second time, especially in those experiments in which the second injection came about 6 hours after the first. It was thought that there would thus be a more continuous and prolonged action of the estrogen. However, the experiments of many others have shown that certain results depend upon a proper sequence and balance between the follicular hormone and the corpus luteum hormone. In the experiments reported here, it is seen that one massive injection of estrogen acting upon the anterior pituitary was sufficient to greatly stimulate the development of the corpus luteum. The excess estrogen may have stimulated peristalsis before its effect was counterbalanced by the increase of progesterone. Excess production of progesterone may have rendered ineffective a second injection of the same amount as first used. It is possible that the second dose should be considerably greater than the first in order to override the increase in progesterone.

We find it unwise at this time to suggest an hypothesis which must include both the tube-locking of ova with 7.5 to 25 R.U. of estrogen and the acceleration of ova with 100 to 500 R.U. There may be not only the direct effect of the estrogen but also factors of the anterior pituitary and the corpus luteum. Some investigators (12, 13, 14, 15, 16, 17) have shown, however, that small doses of estrogenic substances depress the activity of the anterior pituitary, and it is certain that a few massive injections stimulate the anterior pituitary to increase the production of the luteinization factor (18, 19, 20, 21, 22, 23).

SUMMARY

Injections of 100 to 500 R.U. of an estrogenic substance (progynon-B) accelerated the passage of fertilized ova through the fallopian tubes of mice. Results were not always uniform. Ova were frequently found in the uterus, even in the 2-celled stage, 30 to 40 hours after finding the vaginal plug. This is at least 30 hours sooner than usual. Massive injections resulted in the rapid development of the corpora lutea.

These experiments add to our information concerning the factors which control the passage of fertilized ova through the fallopian tubes of mice.

We are grateful to Dr. Gregory Stragnell of the Schering Corporation for generous supplies of progynon-B and proluton.

REFERENCES

1. Whitney, R., and H. O. Burdick: Endocrinology **20:** 643. 1936.
2. Burdick, H. O., R. Whitney and G. Pincus: Anat. Rec. **67:** 513. 1937.
3. Lewis, W. H., and E. S. Wright: Carnegie Inst. Wash. Contrib. Embryol. **25:** 113. 1935.
4. Enzmann, E. V., N. R. Saphir and G. Pincus: Anat. Rec. **54:** 325. 1932.
5. Wislocki, G. B., and F. F. Snyder: Bull. Johns Hopkins Hosp. **52:** 379. 1933.
6. Reynolds, S. R. M.: Am. J. Physiol. **98:** 230. 1931.
7. Reynolds, S. R. M.: Am. J. Physiol. **102:** 39. 1932.
8. Reynolds, S. R. M.: Proc. Soc. Exper. Biol. & Med. **30:** 1165. 1933.
9. Reynolds, S. R. M., and W. M. Firor: Am. J. Physiol. **104:** 331. 1933.
10. Robson, J. M.: J. Physiol. **85:** 145. 1935.

11. Allen, W. M., and S. R. M. Reynolds: Science **82:** 155. 1935.
12. Smith, P. E., and E. T. Engle: Anat. Rec. **42:** 38. 1929.
13. Moore, C. R., and Dorothy Price: Proc. Soc. Exper. Biol. & Med. **28:** 38. 1930.
14. Meyer, R. K., S. L. Leonard, F. L. Hisaw and S. J. Martin: Proc. Soc. Exper. Biol. & Med. **27:** 702. 1930.
15. Leonard, S. L., R. K. Meyer and F. L. Hisaw: Endocrinology **15:** 17. 1931.
16. Spencer, J., R. G. Gustavson and F. E. D'Amour: Proc. Soc. Exper. Biol. & Med. **28:** 500. 1931.
17. Spencer, J., R. G. Gustavson and F. E. D'Amour: Am. J. Anat. **50:** 129. 1932.
18. Hohlweg, W.: Klin. Wchnschr. **13:** 92. 1934.
19. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **32:** 757. 1935.
20. Wolfe, J. M.: Proc. Soc. Exper. Biol. & Med. **32:** 1192. 1935.
21. Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **32:** 1377. 1935.
22. Ellison, E. T., and J. C. Burch: Endocrinology **20:** 746. 1936.
23. Mazer, C., S. L. Israel and B. J. Alpers: Endocrinology **20:** 753. 1936.

DIFFERENTIAL DIAGNOSIS OF PSEUDOCRYPTORCHIDISM AND TRUE CRYPTORCHIDISM

JAMES B. HAMILTON AND GILBERT HUBERT
Departments of Anatomy and Physiology, Albany Medical College, and Department of Pediatrics, Albany General Hospital
ALBANY, NEW YORK

Continued retention of the testicle or true cryptorchidism is often confused with intermittent retention, or 'spastic retraction,' produced by contraction of the muscles enveloping the testicle and cord. It is a common experience among those who treat cryptorchidism to receive many cases of pseudocryptorchidism which have been diagnosed as true. Of 16 patients referred by pediatricians to the authors, only 6 were actually cryptorchid. The English surgeon McLellan (1) wrote recently, "No less than 12 patients with 'undescended testis' were sent to me by practitioners who all had special experience in the examination of children, and in all I was able to get the testes satisfactorily into the scrotum."

In view of the frequency of confusion between physiological and anatomical cryptorchidism, the difficulties in diagnosis unless special precautions are taken, and the difference in the mode of therapy which may be chosen in these states, it has seemed advisable to present a method devised by one of us (J.B.H.) to differentiate the true from the false cryptorchidism.

METHOD

The rationale is that of obtaining relaxation of those muscles whose contraction has caused retraction of the testis; if this does not in itself result in return of the testis to the scrotum, manual palpation is also employed. Such relaxation of cremasteric and other muscles is obtained by a general method of approach and by direct application of heat to the groin, scrotum and perineum, the patient lying with legs apart.

The general procedure is first to place the subject as much at ease as possible. Apprehension is partially relieved by explaining the painlessness of the method, followed by a casual observation and brief palpation for the testes. A hot water bag is then put upon the scrotum, inguinum and perineum, and the patient covered with blankets sufficient to assure warmth. Children in particular are reassured by the painlessness of the first mock examination and later, after the heat has been applied for 30 minutes, they can be examined thoroughly, usually without arousing their fears. The bag is wrapped with a single layer of flannel so that the rubber does not come in contact with the skin. The temperature of the water in the bag is maintained at about 115°F.

The specific procedure of thermal applications warms the muscles whose contraction cause retraction of the testis and interfere with the return of the

testis normally or even when aided by manual pressure. Heating results in relaxation of these muscles; hence, the testis may re-enter the scrotum, either spontaneously or following increase in the abdominal pressure. When spontaneous descent does not occur, palpation is employed. If the testis cannot be passed into the scrotum by gentle manipulation when the muscles are relaxed, the testis is considered to all intents and purposes to be truly cryptorchid.

Precautions should be observed to avoid exciting the patient or inciting the active cremasteric reflexes. The unexpected entrance of the nurse or other person is to be deprecated. Further, a stroke against the thigh, or, more com-

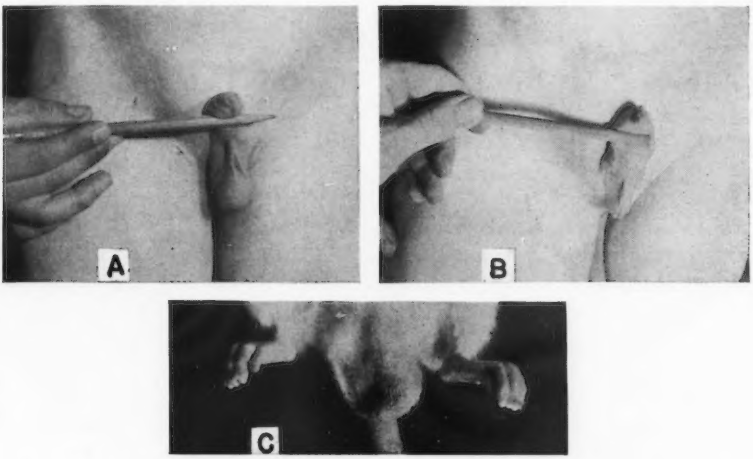


Fig. 1. Influence of presence of testis on scrotal size. *A*, One-sided scrotal development in true unilateral cryptorchidism. *B*, Rudimentary scrotum in true bilateral cryptorchidism. *C*, Scrotal atrophy in a rat following experimental unilateral cryptorchidism.

monly, the contact of the examiner's cool fingers against the abdomen of the patient may elicit a reflex ascent of the testis.

Observation and the patient's history are also valuable in defining pseudocryptorchidism. If the condition be merely one of intermittent retention, the scrotum is generally well-developed. This is in contrast to the one-sided scrotal development seen in true unilateral cryptorchidism (fig. 1 *A*) or the rudimentary scrotum of the bilateral condition (fig. 1, *B*). The absence of associated inguinal hernia is characteristic of the spastic case. Still other evidence is afforded by noting whether the retention is bilateral as is to be expected in spastic retractions. The patient's history, obtained either directly or in the case of young subjects from parents or nurse, reveals whether or not the testis has been observed to be in the scrotum from time to time. Confirmation of intermittent retention is often gained from the history.

RESULTS

Sixteen cases were referred to us in which a diagnosis of cryptorchidism had been made by ordinary methods of examination. When the necessary pre-

cautions had been taken, these patients were observed preliminarily and then a second time after the application of heat to the groin and scrotum. In 6 cases the cryptorchidism was real, in 10 false. Thus, this method permitted identification of 62.8 per cent of the cases referred as cryptorchid to be instances of spastic retention.

It was noted that although the application of heat permitted easier descent in cases of spastic retraction, the cremasteric reflex was not entirely negated, for gentle stroking of the inner surface of the thigh was often sufficient to induce re-ascent of the testicle. In several cases, however, in which the cremasteric reflex had been elicited after heating, the testis returned spontaneously to the scrotum immediately or within a few moments.

The value of observing the scrotal development was apparent. That side of the scrotum in which the testis was continually or intermittently descended was well-formed and of approximately normal size. In all cases in which the testis was continually absent from the scrotum, the scrotum on that side was small and underdeveloped. This small size was particularly apparent in unilateral cases of true cryptorchidism, where the scrotum on the cryptorchid side was by contrast notably smaller. Proof that the small size of the scrotum was correlated with the absence of the testicle was obtained from animal experiments. In rats one testis was rendered cryptorchid by suturing it to the abdominal wall and as a further measure to prevent its return to the scrotum, the inguinal canal on that side obliterated by ligature. The other testis was allowed to remain in the scrotum. Figure 1, *C* shows the distinct difference between that half of the scrotum which contained a testis and the other side, from which the testis had been removed. This distinction extended into the microscopic structure of the scrotum (2).

It is interesting to note that in the same child the pseudocryptorchidism might be encountered on the right side one day, whereas at another time the left side, but not the right side, would exhibit the condition. Save for such exceptional cases, however, the pseudocryptorchidism was generally found to be bilateral.

DISCUSSION

The importance of differentiating the true from the false cryptorchidism lies not alone in relieving the anxiety of patient and parents, but in establishing the course of treatment to be followed. If the testis be retained but intermittently, endocrine or surgical treatment is generally held to be unnecessary, although there is the possibility that treatment of this condiion simply with hormonal substance may suffice to assure continued scrotal retention. This possibility is under investigation.

In true cryptorchidism there would seem to be little gained from a policy of waiting and watching. The finding that a large proportion of retained testes are only intermittently so raises the question as to the reliability of the data claiming a high percentage of spontaneous descent in cryptorchidism (3). May not many of these cases of spontaneous descent have been in reality but physiological retention? Wangensteen (4) has already criticized Drake's (3) figures of cryptorchid cases which showed spontaneous descent as being much

too high for normal incidence of cryptorchidism. Such a criticism gains support in view of the present report in which 16 patients, referred by pediatricians as cryptorchid, were shown by the above-described methods to be but spastic retractions. Such adequate treatment was not used in the many cases listed as descending spontaneously. It seems justifiable to insist that future cases which are to be classified as descending spontaneously must be examined with at least the precautions outlined in this article to eliminate inclusion of testes retained intermittently.

Failure to differentiate between false and true cryptorchidism may also explain the divergent findings reported by various workers who have used anterior pituitary-like substances. Pseudocryptorchidism, which can easily pass for true cryptorchidism as shown by this paper, would be expected to give good results with any treatment, for the intermittently-retained testis is, as its name implies, subject to spontaneous descent. Harris (5) describes a case in which the testicle descended within 3 hours following a single injection of 100 R.U. of gonadotropic material. It seems worthwhile in future treatment and observation of cryptorchidism, therefore, to check by such methods, as given above the exact nature of the retention, that is, whether the retention be intermittent or continuous.

Thus, differential diagnosis of false and true cryptorchidism permits relief of unnecessary anxiety in the former instance and immediate treatment in the latter. The dangers of continued cryptorchidism are well-recognized and it is our belief that treatment should be insisted upon in view of such possible sequelae as hernia, trauma, adhesions, hydrocoele, tortion, testicular degeneration, aspermatogenesis, and malignancy. The incidence of the complications in cryptorchidism and the choice of treatment will be reserved for a more complete discussion with but a brief statement now that endocrine treatment is valuable *1)* in bringing about descent in some cases; *2)* in identifying those testes which cannot be expected to descend at puberty; hence, earlier treatment can be instituted before the above-mentioned complications occur; *3)* in cases in which orchiopexy is necessary, in greatly facilitating surgery by developing and elongating cord elements; and *4)* in reducing post-operative stretching and tension on testicle and adnexa.

SUMMARY

A technique is described of direct application of heat to scrotum, inguinum and perineum for the purpose of obtaining relaxation of the muscles which produce spastic retention. This method, plus the precautions, general procedures and observations enumerated, permits differential diagnosis of intermittent and continuous retention of the testicle. Employment of this technique reveals the large percentage of pseudocryptorchid testes that are considered true cryptorchidism by usual tests, even by physicians experienced in the handling of children. Of the 16 cases referred to us by pediatricians all but 6 presented a condition of spastic retraction.

The high incidence of pseudocryptorchidism masquerading as true cryptorchidism leads one to question the large percentages of 'spontaneous descent'

in supposedly true cryptorchidism. Further, it may partially explain the discrepancy in the results of various authors in the treatment of cryptorchidism with endocrine preparations.

It is suggested that some such precautions as outlined in this paper be employed in future designation of true cryptorchidism, since such procedures seem necessary to eliminate pseudocryptorchidism, and since it would provide standardization of a variable phenomenon so that the results of different groups of workers might be compared.

REFERENCES

1. McLellan, E.: Lancet **1:** 999. 1936.
2. Hamilton, J. B.: Proc. Soc. Exper. Biol. & Med. **35:** 386. 1936.
3. Drake, C. B.: J.A.M.A. **102:** 759. 1934.
4. Wangensteen, O. H.: Ann. Surg. **102:** 875. 1935.
5. Harris, F.: Am. J. Surg. **27:** 447. 1935.

TREATMENT OF SEXUAL UNDERDEVELOPMENT WITH SYNTHETIC MALE HORMONE SUBSTANCE[1]

JAMES B. HAMILTON[2]

From the Departments of Anatomy and Physiology, Albany Medical College, and Albany Hospital

ALBANY, NEW YORK

This report of a single case is deemed worthwhile because of *1)* the lack of reports of similarly treated cases, *2)* the very limited amount of synthetic male hormone that was available for this investigation, *3)* the extensive study made of this individual before and after treatment, and *4)* the wide bearing of these findings upon endocrine treatment of hypogenitalism, cryptorchidism, impotence, migraine, acne and menopausal symptoms.

History and Examination. The patient was a 27-year-old white male medical student whose chief complaints were headache in right frontal and temporal regions, attacks of malaise, hot flushes, proneness to fatigue and social stigmata due to feminine aspect and high voice. In 1933 the patient received 3 injections of 1 cc. antuitrin-S weekly for a 3-week period, followed by 2 weeks of rest; this was continued for 3 months with but slight alleviation of symptoms. Save for similar menopausal symptoms of flushes and headaches in the mother, the family history was irrelevant. The patient had had measles, influenza complicated by pneumonia, chicken pox and scarlet fever before the age of 15. The organ systems except the endocrine, reproductive, osseous and integumentary were essentially normal.

The general body picture was that of a pre-puberal castrate with a feminine emphasis in the wide hips, genu valgum, girdle distribution of fat, protruding mammae, and retarded development of larynx, genitalia and hair (fig. 1, *A*). The long limb bones were proportionally longer than the other bones. The epiphyseal union was not complete in the ulna or radius. The jaw, facial eminences and digits might be considered to be of a hypopituitary type and in contrast to the condition encountered in acromegaly. Figure 1, *A,* shows the receding jaw, non-prominence of facial eminences, and the long, tapering digits. The proximal metacarpals of the index and second fingers were of a greater length and diameter than those of the other two fingers. Hair was sparsely distributed in the axilla and in a feminine manner on the pubis. There was no hair on the abdomen, chest or face, save on the upper lip which was shaved more from desire than necessity about once every 10 weeks. Large dark circles were seen beneath the eyes. The genitalia (fig. 1, *B*) were somewhat smaller in size and degree of development than those of a small boy of 3 or 4 years of age. The penis was 2.5 cm. long and 1.2 cm. in diameter, with a prepuce not clearly differentiated from the glans. The urethal orifice was exceptionally small. The scrotum was a flat band-like structure without rugae or pen-

[1] This study was aided by a fund supplied by Dr. George Walker.

[2] The author wishes to express his appreciation to Doctors Eldridge Campbell, Judson Gilbert, John Heslin, Harry Tebbutt, Joseph Schwind, and Harold Himwich for their cooperation and interest in the carrying out of this study.

dulousness, its wall thin and not deeply pigmented. The testes and epididymides were not identified with certainty. The patient stated that infrequent erections but not ejaculations had occurred. A prostate could not be identified by rectal palpation save that 2 small pea-sized lumps could be felt beneath a pad of edematous tissue. The mammae were large with light pink nipples and areolae. Only adipose tissue could be palpated.

Roentgenograms of the pituitary disclosed a sella turcica within low normal limits (12 mm.). Repeated assays of the urine for gonadotropic substance gave negative results in the rabbit pregnancy test.

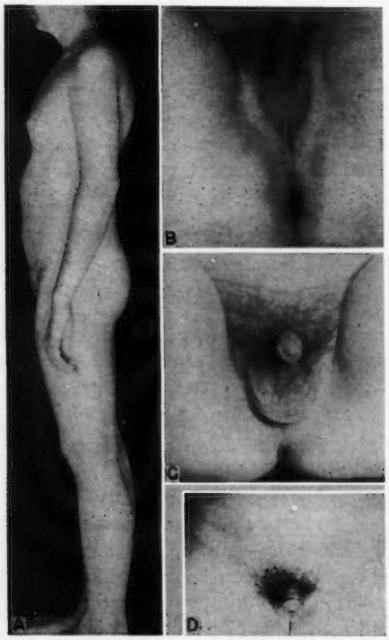


Fig. 1. *A* and *B*, Patient before treatment. Note sexual and laryngeal underdevelopment, gynecomastia. *C* and *D*, Same patient after 3 weeks treatment with male hormone substance, testosterone acetate.

The larynx was shown to be small on laryngoscopic and x-ray examination, indistinguishable in external profile, and lacking in the growth changes produced in the male larynx at puberty. The voice was very high-pitched and of small volume. The nasal mucosa exhibited no congestion, swelling, or secretion in areas which have been shown in monkeys and in humans to be controlled by male hormone substance (1).

The patient was intelligent, industrious, trustworthy, prone to anxiety, emotionally unstable, concerned about and feeling somewhat inferior because of his condition. No feminine traits were discernible. He was engaged to be married.

The basal metabolic rate was —17 and —19. Blood and urine constituents were present in essentially normal amounts. Perhaps noteworthy was the absence of creatinuria. Ten tests made over a period of 3 years gave an average daily level of 1.1 mg.

Treatment. The patient was given a total of 550 mg. of testosterone acetate[3] in 14 injections, 3 injections per week, for a period of 1 month. Peanut oil was selected as the vehicle, since the type and amount of oil is significant (2).

Injections were subcutaneous and intramuscular, in arm or in buttock. At the end of these injections the patient received oil which, unknown to him, did not contain the potent male hormone substance he had been receiving. When he became thoroughly aware of the absence of response, all injections were stopped for 1.5 months. Injections were begun again 2 times a week, with a smaller dosage of 5 mg. of testosterone propionate in peanut oil, since the propionate compound has been shown to be absorbed more slowly than the acetate (2). After 10 injections of 5 mg. each, the dosage was increased to 20 mg. of testosterone propionate given twice weekly.

RESULTS

First Period of Treatment. The effect of the hormone administration was seen within 60 hours after the first injection, when the patient experienced pronounced penile erections. After 6 days erections occurred repeatedly and continued throughout the month, at times reaching a state of priapism. Despite the small size of the penis the patient felt conspicuous because of the erectile condition. The left side of the scrotum contained a mass in which testis and epididymis were not distinguished. The scrotum appeared to be somewhat thicker and of a darker color, the glans penis more pink.

Within 2 weeks the scrotum became much larger, rugose, pendulous, and more pigmented. The mass in the left side of the scrotum enlarged until a well-formed epididymis could be differentiated from a tiny atrophic testis. The penis increased in both length and diameter. The larynx was congested; the thyroid was felt to be larger.

After 3 weeks the areolar tissue became more darkly pigmented, somewhat rugose and tender to deep pressure. The nipple was more prominent and beneath it appeared a rather firm area. On rectal examination the prostate was felt to be heart-shaped and of moderate thickness. Both testes and epididymides were palpable in the scrotum. Figures 1, *C* and *D* show the increased length and diameter of the penis, and the pendulousness of the scrotum. The urethral opening was also enlarged.

The hot flushes disappeared entirely throughout the period of treatment. One moderately severe migraine attack occurred. The patient was more energetic than usual, self-assured and in higher spirits. There was some question as to a lowering of the pitch of the voice. Small hairs appeared on the chest and in a masculine distribution above the pubis. Periods between shaving of the upper lip were shortened to 2 weeks. The dark circles beneath the eyes became lighter in color.

Period of Withdrawal. Five days following the last injection a series of 4 hot flushes were experienced and the erections became infrequent. By 11 days there was a moderately severe attack of migraine, repeated hot flushes, lassitude, some loss of self-assurance, and some huskiness of the voice. By 16 days an acneform eruption was seen on back and chest, which progressed to include

[3] Testosterone acetate and testosterone propionate (Perandren) were furnished through the courtesy of the Ciba Company.

the face and neck. Hot flushes and migraine occurred as before treatment. There was some decrease in prostatic, epididymal and scrotal size, but less regression of penile growth. Mammary tenderness persisted and a specimen which was taken for biopsy seemed to resemble female mammary structure in gross characteristics, but in histological examination was seen to contain lacteriferous ducts without alveolar tissue.

The acne gradually disappeared as the period of hormone withdrawal lengthened to 1½ months. The dark circles returned beneath the eyes.

Second Period of Treatment. A smaller dosage of 5 mg. of testosterone propionate was employed twice weekly. The phenomena observed in the first period of treatment were again encountered but in lesser degree. Erections reappeared, hot flushes disappeared; migraine attacks became less severe. Genital growth was resumed. The acne returned at the onset of the injections, but disappeared as the treatment continued. After 10 injections, the dosage was increased to 20 mg. administered twice weekly. Growth became more rapid. The patient reported more energy and self-assurance.

DISCUSSION

The course of this study has permitted *1)* recognition of the effects of synthetic male hormone therapy in man; this patient represented a biologically-controlled subject due to an extreme hypogonadal condition: *2)* differentiation of results due to injected male hormone from those due to bodily-produced male hormone, since *a)* the patient was physiologically castrate, and *b)* the growth changes followed injections, regressed upon cessation of injections and reappeared after resumption of injections: *3)* distinguishing of effects of the injected hormone from mentally-induced reactions, *a)* since when the patient received impotent substance, which he thought was potent, a lack of response appeared and was recognized by the patient, and *b)* by virtue of intelligence and medical training, the patient carefully analyzed and separated these effects as much as possible.

The data concerning the response of this patient is relevant to several clinical conditions which, theoretically at least, can be treated either by anterior pituitary-like substances (gonadotropic material extracted from pregnancy urine, such as antuitrin-S or follutein), or by gonadal substances, which in the male can be testicular extracts or synthetic material. Without a lengthy discussion of the relative merits of gonadotropic and gonadal substances (3), this patient will serve as an example of the results being obtained with synthetic male hormone (gonadal) substances in several of the following conditions, until our cases under study at present can be compared with those treated by pregnancy urine extracts.

Male Hypogonadism. Pregnancy urine extracts and male hormone substances develop secondary sex characteristics, but in regard to spermatogenesis can be expected to do no more than maintain an already existing spermatogenesis. Even such maintenance has been proven only in animals (4, 5, 6). For the induction of sperm production it seems likely that a gametokinetic substance, such as might be obtained from menopausal or castrate urine, would be necessary.

Since presumably the effect of gonadotropic substances on the genitalia (7) is

only by stimulation of the gonads to produce gonadal material and must, therefore, depend upon a responsive testis, it is interesting to note that the patient reported here had responded but slightly to over 30 injections of 1 cc. antuitrin-S.

Cryptorchidism. Descent of the testes with pregnancy urine extracts has been obtained by many workers, an effect believed by the author (3) to be due to the male hormone produced upon stimulation of the testis by gonadotropic material. The use of anterior pituitary-like substances is subject to the criticism that they depend upon a responsive testis, are but extracts, and may have a wide variety of effects besides that desired. An evaluation of the benefits and limitations of male hormone substances as determined by studies in monkeys and humans has been written (8), along with a partial analysis of the processes incited by male hormone in producing descent of the testis.

Impotence. It is difficult to separate the mental from the physical aspects in incapacities to obtain penile erection. This renders all the more significant the finding in immature animals and in children as young as 18 months that erections have been induced and in some instances approach a condition of priapism following injections of the male hormone substances, testosterone acetate and testosterone propionate (9). Striking, too, are the erections in the present case and the finding (9) that a man, impotent for 8 years, can carry on intercourse after he received injections of male hormone substance, but not after he received injections of the oil vehicle alone—even though he believed the mock injections to be potent. Induction of erections has been reported in 2 hypogonadal cases receiving male hormone extracts (10), and in cryptorchid children receiving pregnancy urine extracts (11) (which presumably stimulate the testis to produce made hormone).

Acne. Some degree of success has been reported in the treatment of acne with gonadotropic (12) and estrogenic (13) material. It is interesting to note that this patient, who had never shown evidence of the presence of male hormone or of acne, did not develop the condition when large amounts were injected in the first period of treatment, but acquired acne in the period following cessation of treatment, when assumedly the male hormone content fell to a fairly low level (hot flushes had appeared). Toward the end of the withdrawal period the acne tended to disappear, but returned when small amounts of male hormone were administered and then disappeared when a higher level of male hormone substance was attained. On this suggestive evidence a small group of boys complaining of severe acne during puberty has been treated with testosterone propionate with disappearance of the acne. A long post-treatment study and more cases are necessary, however, before the value and contraindications of this treatment can be ascertained.

Hot Flushes and Migraine. The prompt disappearance of hot flushes when the patient was under treatment and the equally rapid reappearance upon cessation of treatment may suggest a study of male hormone treatment of menopausal conditions. Extreme caution is urged, however, for male hormone substances have a pronounced masculinizing effect in the female (14), and will inhibit pituitary activity (15) in rodents and menstruation in both monkey and human (8). The results obtained with gonadotropic (16) and estrogenic (17) substances in certain types of migraine plus the alleviation seen in the patient during treatment may suggest male hormone treatment of this condition. In the female, at least, much too little is known about the influence of male substances to risk haphazard use.

Voice. The possibility of lowering of the pitch of the voice is intriguing and in this patient is a vital point, since he feels that fairly authoritative vocal expression would aid him considerably in the contacts incident to the practice of medicine.

Miscellaneous. The changes in the color of the region below the eye are incom-

plete evidence of control exerted over this region by gonadal hormones and is in agreement with the changes noticeable in some women during various parts of the menstrual cycle. It recalls the facial sexual skin in monkeys.

The mastitis brought to mind the transient mastitis often encountered in boys at the time of puberty.

Mental Changes. Definite relationship of male hormone level to mental attitude is difficult because the patient realized that an attempt was being made to help him, and more important, he realized the progress he was making physically. This knowledge plus the number of other accessory influences make definite correlation precarious. The subject attempted, however, with admirable aloofness and clarity to study the changes in himself and was convinced that he was more energetic, virile and self-assured when under treatment. The increase in these qualities was vouchsafed by associates. The subject complained during cessation of treatment that his attempts to combat recession were futile and he believed that his buoyancy of spirits was not confined to physical betterment. It will be worthwhile to note the results of psychologists and psychiatrists in the use of this material in adolescence and in such patients as male perverts.

Finally, it should be noted that no deleterious effects were observed on the blood or urine constituents of the patient, a fact confirmed by similar tests in other patients receiving testosterone acetate and propionate (8). Nevertheless, the period of treatment in this patient has been short. Further observation and many additional cases must be investigated before generalizations can be made.

SUMMARY

Synthetic male hormone substances were administered to a 27-year old hypogonadal male, 'physiologically castrate' from birth, who provided an excellent subject for evaluation of the effect of these materials. The hypogonadism, cryptorchidism, impotence, hot flushes, migraine, and mental attitude were treated with some degree of success. Brief discussion is given of the relation of these results in this clear-cut case to the subject of male hormone treatment, with especial reference to cryptorchidism, hypogonadism, impotence, menopausal symptoms, migraine and acne. The possible influence of made hormone upon the pitch of the voice, the area of pigmented skin under the eye and the mental attitude is mentioned.

REFERENCES

1. Hamilton, J. B.: In Press.
2. Parkes, A. S.: Lancet **2:** 674. 1936.
3. Hamilton, J. B.: Proc. Am. Physiol. Soc. P. 69. 1937.
4. Walsh, E. L., W. K. Cuyler, and D. R. McCullagh: Am. J. Physiol. **107:** 508. 1934.
5. Nelson, W. O.: Science **84:** 230. 1936.
6. Hamilton, J. B.: Proc. Soc. Exper. Biol. & Med. **35:** 386. 1936.
7. Sexton, D. L.: Endocrinology **20:** 781. 1936.
8. Hamilton, J. B.: Unpublished data.
9. Hamilton, J. B.: In Press.
10. Koch, F. C.: J. Urol. **35:** 382. 1936.
11. Dorff, G.: J. Pediat. **8:** 704. 1936.
12. Lawrence, C. H.: New England J. Med. **212:** 1213. 1935. J.A.M.A. **106:** 983. 1936.
13. Van Studdiford, M.: Arch. Dermat. & Syph. **31:** 333. 1935.
14. Hamilton, J. B.: Anat. Rec. **67:** (Suppl.). 22. 1937.
    Hamilton, J. B., and J. M. Wolfe. Proc. Soc. Exper. Biol. & Med. **36:** 465. 1937.
15. Wolfe, J. M., and J. B. Hamilton: Proc. Soc. Exper. Biol. & Med. **36:** 307. 1937.
16. Pardee, I. H.: Arch. Int. Med. **33:** 174. 1919.
    Moffat, W. M.: J.A.M.A. **108:** 612. 1937.
17. Blakie, N. H., and J. C. Hossack: Canad. M.A.J. **27:** 45. 1932.
    Critchley, M.: Practitioner **133:** 54. 1934.

TESTIS HORMONE IN RELATION TO AGE

CHARLES W. HOOKER
From the Zoology Laboratory, Duke University
DURHAM, NORTH CAROLINA

It is widely held that variations in the state of secondary sex characters in the male are due to variations in the amount of hormone produced by the testis, the transformations of puberty being regarded as a manifestation of an abrupt and great increase in testis hormone secretion and senile changes being attributed to diminution or cessation of this secretory activity. There are, however, few data on hormone production at different stages in the life cycle. Womack and Koch (1), without citing values for individual animals, report the highest concentration of testis hormone in fetal and young calves, with a progressive decrease thereafter. Funk, Harrow, and Lejwa (2) report less comb-growth promoting activity in the urine of old men than in the urine of young adults. Womack and Koch (3) state that no hormone occurs in the urine of boys under 10 years of age, while after 10 years its concentration becomes as great as in adults. Bühler (4) also reports the absence of the hormone in urine before puberty, while between puberty and 40 years there are 2 bird units per 24 hours, between 40 and 60 years less than 1 B.U., and after 60 years none.

The present report deals with an attempt to determine the amount of male hormone in the bull testis at different ages, and more particularly to determine whether changes in its production accompany the marked alterations in masculinity which characterize puberty and senility.

MATERIALS AND METHODS

A series of 27 bulls ranging in age from 1 month to 15 years has been studied. From each animal a 24-hour specimen of urine was obtained. At the conclusion of the 24-hour period the animal was either slaughtered or castrated. From each pair of testes a fragment was preserved for histological study and the testes placed on CO_2 ice until they reached the laboratory.

The testes were stripped of all coverings, weighed, ground, and extracted by the method of Gallagher and Koch (5) through the cold acetone stage. The urines were extracted by the method of the same authors (6)through the alkali-extracted ether phase. The extracts were essayed on White Leghorn capons by the method of Gallagher and Koch (7) and on rats by the prostate and seminal vesicle cytology test (8). For reason of their greater sensitivity, the rat assays were used exclusively in the younger animals, from which a small quantity of hormone was expected.

RESULTS

Beginning at the age of 1½ months, when the total hormone content of both testes is 1.0 bird unit, there is a gradual increase up to 1½ or 2 years (table 1). Thereafter the hormone content rises sharply, attaining a peak of 125.1 B. U. at 5 years, followed by a progressive and regular decline to a value of 25.3 B. U. at 15 years. The values obtained for B14 at 6 months and B6 at 18 months are departures from the general trend. Whether these discrepancies are significant is uncertain. The low values for B31 and B24 are undoubtedly

TABLE 1.

Number	Weight lb.	Age	Wt. Testes grams	Testis Assay B.U.
B9	110	1½ mo.	9.0	1.0
B8	132	2 mo.	12.7	1.2
B7	180	3 mo.	32.5	3.0
B20	166	3 mo.	23.0	3.5
B10	150	4 mo.	33.6	3.0
B23	219	5 mo.	35.5	4.0
B14		6 mo.	99.3	9.0
B28	244	7 mo.	75.0	4.5
B31	230	7½ mo.	37.5	1.0
B22	314	8 mo.	149.0	2.5
B32	240	9 mo.	37.0	1.0
B29	330	9 mo.	132.0	5.0
B24	210	9½ mo.	72.0	3.0
B15		10 mo.	168.0	6.7
B5	286	10½ mo.	163.6	8.3
B18		14 mo.	221.0	7.0
B25	402	14 mo.	218.0	8.0
B26	542	15 mo.	323.5	10.5
B6	706	18 mo.	322.0	35.1
B27		18 mo.	474.5	12.0
B3		2 yr.	408.0	16.0
B30	447	2 yr.	246.5	10.0
B12	666	2½ yr.	565.0	29.4
B11	1500	5 yr.	681.0	125.1
B17	1580	7 yr.	610.3	64.7
B21	1320	13 yr.	530.5	34.0
B16	1410	15 yr.	436.0	25.3

due to the poor condition of the animals resulting from infectious diarrhea.

The presence of motile spermatozoa in the epididymis was selected as the criterion of sexual maturity; and despite certain objections it is probably a satisfactory index, since motile spermotozoa were always found after the age of 10 months and bulls are known to be capable of reproducing at one year and often earlier. Of the animals in the group under 10 months only B22 showed spermatozoa, this at the age of 8 months.

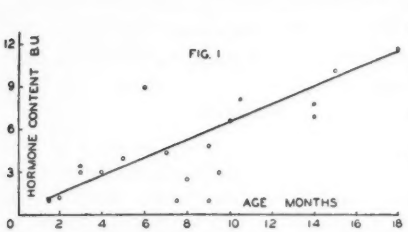
There is no indication of a marked rise in hormone production preceding, accompanying or immediately subsequent to sexual maturity. As shown in figure 1, the rate of production of the hormone (measured by the quantity of hormone extractable in the testes) shows a gradual and regular increase with age throughout the first 18 months. The change in rate of production is so uniform that is is impossible from inspection of the curve to ascertain at what point the animal attains sexual maturity.

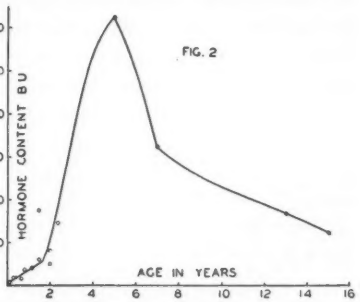
Commencing shortly after 2 years there is a much more rapid increase in hormone production, and this change in rate is quite sharp (fig. 2). The change, it will be noted, comes not at puberty but more than a year later. The rate of production continues to increase to 5 years; after 5 years hormone production decreases with age, reaching the level of 25.3 B. U. at 15 years.

An unexpected finding was the failure to obtain the comb-growth factor in any of the urines. Womack and Koch (3) have reported failure with calf's urine; but in the present investigation none of the urine extracts exhibited any demonstrable activity even though the daily dose to the test animal attained the equivalent of nearly 2 liters of crude urine. In most cases the daily dose to the test animal was the equivalent of one-tenth of the 24-hour sample.

DISCUSSION

The very gradual and uniform increase in hormone production throughout the first 18 months of life in the bull points unmistakably to the generalization





that puberty, at any rate in this species and by the chosen criteria, is not a period of great and rapid change in hormone production by the testes, but a period during which tissues influenced by the hormone undergo change even in the absence of coincident increase of stimulation from the testis.

This concept is at variance with a prevailing characterization of puberty as a period in which there is a great alteration in both the internal secretory activity and gametogenic function of the gonad. Hansen's (8) finding that more hormone is required to repair castration changes than to maintain the normal state in the adult has been cited to indicate that a greater quantity of hormone is necessary for the transformation into the sexually mature state than for its maintenance. In view of the failure of hormone production to follow this principle one is led to postulate that at puberty the tissues acquire a capacity to react to a quantity of hormone to which they have been hitherto refractory. Such a view is tenable on the basis that response to a hormone follows only when two conditions are met: the hormone must be available in sufficient quantity, and the tissues must be in a state capable of responding.

There are other instances in which age is an important factor in conditioning response to a hormone. The reaction of immature rats to gonadotropic substance is heightened as the animals approach puberty (9, 10, 11). Estrin

will produce female feathering in the male fowl after the age when sex dimorphism in plumage is normally established, but is ineffective in younger males (Juhn, Gustavson, and Gallagher, 12). These authors obtained stimulation in the gonoducts of just-hatched chicks to small doses of sex hormones, while Willier and his co-workers (13) found that enormous doses were required to elicit the same response in chick embryos examined on the 19th day of incubation. More pertinent to the present discussion are the data of Freud (14) which indicate a definite increase in reactivity to the male hormone on the part of the sex accessories in rats at about the time of puberty. Freud does not state the ages of his animals nor does he emphasize the point indicated here.

The failure to obtain male hormone in the urines examined in this study contrasts somewhat with the report of Koch (15) that bull urine possesses androgenic activity in amounts less than 1 bird unit per liter. However, Koch gives no detail concerning the method he employed and the difference may well represent an improvement in the method of extraction. It seems certain that the amount excreted by the bull is markedly less than that by man, and perhaps represents a qualitative difference in the metabolism of the hormone by the two species, since the concentration of androgenic activity in human testis is essentially the same as in bull testis (16).

SUMMARY

From a series of bulls ranging in age from 1 month to 15 years the testes and 24-hour urines were extracted for male hormone. Assays of the testis extracts show that the hormone content increases slightly but progressively to the age of about 2 years, when there is a sharp increase which is continued to 5 years. After 5 years the hormone content gradually decreases.

Since puberty is attended by no sharp change in the rate of hormone production by the testes, the hypothesis is advanced that at this age the tissues acquire a capacity to respond to a quantity of hormone previously ineffective.

I should like to acknowledge indebtedness to Dr. Bert Cunningham under whose direction this work was done; to Dr. C. D. Grinnells of North Carolina State College who located the animals studied; and to Dr. Harold Cummins who has read and criticized the manuscript.

REFERENCES

1. Womack, E. B., and F. C. Koch: Endocrinology **16:** 267. 1932.
2. Funk, C., B. Harrow and A. Lejwa: Proc. Soc. Exper. Biol. & Med. **26:** 569. 1929.
3. Womack, E. B., and F. C. Koch: Endocrinology **16:** 273. 1932.
4. Bühler, F.: Ztschr. f. d. ges. exper. Med. **86:** 650. 1933.
5. Gallagher, T. F., and F. C. Koch: J. Biol. Chem. **74:** 495. 1929.
6. Gallagher, T. F., and F. C. Koch: Endocrinology **18:** 107. 1934.
7. Gallagher, T. F., and F. C. Koch: J. Pharmacol. & Exper. Therap. **40:** 327. 1930.
8. Hansen, I. B.: Endocrinology, **17:** 163. 1933.
9. Smith, P. E., and E. T. Engle: Am. J. Anat. **40:** 159. 1927.
10. Corey, E. L.: Physiol. Zool. **3:** 379. 1930.
11. Wiesner, B. P.: J. Physiol. **75:** 39. 1932.
12. Juhn, M., R. G. Gustavson and T. F. Gallagher: J. Exper. Zool. **64:** 133. 1932.
13. Willier, B. H., T. F. Gallagher and F. C. Koch: Physiol. Zool. **10:** 101. 1937.
14. Freud. J.: Biochem. J. **27:** 1438. 1933.
15. Koch. F. C.: Physiol. Rev. **17:** 153. 1937.
16. Hooker, C. W., and J. S. Raper: Unpublished data.

THE BIOLOGICAL EFFECTS OF THYMECTOMY

ACCRUING RETARDATION IN GROWTH AND DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMECTOMIZED RATS[1]

NATHAN H. EINHORN[2] AND LEONARD G. ROWNTREE
From the Samuel Bell, Jr., Laboratory of the Philadelphia Institute for Medical Research, in the Philadelphia General Hospital
PHILADELPHIA, PENNSYLVANIA

In a previous communication, we reported that thymectomy in successive generations of parent albino rats of the Wistar strain resulted in accruing retardation in growth of the offspring of the second (F_1) and third (F_2) generations (1, 2). The purpose of this paper is to supplement the first report with additional data, and to add observations on animals through the sixth (F_5) generation.

METHODS OF EXPERIMENTAL PROCEDURE

Six pairs of albino rats (Wistar strain) of known parentage were selected. Four pairs were thymectomized by the technique previously described, and the remaining 2 pairs, used as controls, were subjected to a similar operative procedure, except that the thymi were not removed (1). The rats were mated, designated by numbers, and their offspring studied. Data on the somatic development were recorded daily, and the weights of the rats twice weekly up to the age of 70 days. All of the animals in one litter or part of a large litter of each pair of parent animals were operated upon, mated, and set up as parents of the next generation.

During the course of this investigation, and up to Jan. 1, 1937, a total of 105 rats have been thymectomized; of these, 27 or 26 per cent died during or immediately following the operation. The earliest age at which the young were thymectomized was 17 days, the latest 24 days, the determining factors being the condition of the animals and whether they could be successfully weaned. All of the surviving rats were weaned between the ages of 21 and 28 days, depending upon their condition and strength. Occasionally weaker animals were returned to their mothers post-operatively for a few days before being weaned. All rats were housed and fed under the conditions previously reported (1).

The control data used in this study have been obtained from a study of 3 groups of rats: *A),* from the data obtained at the Wistar Institute and summarized by Donaldson (3); *B),* from the control series of the Philadelphia Institute for Medical Research; *C),* from the operative control group whose parents were subjected to the surgical technique followed in thymectomy,

[1] This work has been aided by a Grant from the Penrose Fund of the American Philosophical Society.
[2] Part of thesis studies submitted to the Faculty of the Graduate School of Medicine of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Medical Science, Sc.D. (Med.)

without ablation of the gland. A total of 208 animals in 24 litters were born from the second (F_1) to the fifth (F_4) generation of this operative control group. Of this number 183, or 87 per cent survived the 70-day period of observation. It was noted that the growth and development of all of these litters were essentially the same, irrespective of the generation. The data have therefore not been segregated by generations, and the figures presented in table 1 are based upon the entire group of 208 animals. The weight curves of

TABLE 1. COMPARISON OF SOMATIC DEVELOPMENT IN CONTROL RATS

Strain	Average no. in liter	Ears open	Incisor erupt	Hair obscures genitalia	Eyes open	Testes descend	Vagina open	Comment
Wistar Institute	6.1	2½–3½	8–10	16	14–17	40	72	Fed on a varied breeder's diet.
Phila. Institute for Medical Research	5.0	2½–3½ (3)	8–10 (9)	16 (16)	14–17 (15.5)	31–40 (38)	55–62	Fed on an adequate stock diet.
Operative Controls	6–12	2–3 (2.9)	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)	Fed on an adequate stock diet.

* Figures in parentheses represent average values, in days.

the 3 series of control animals are almost identical. The weight curves of the operative controls and the normal controls of the Philadelphia Institute coincide closely throughout the entire period of observation. In table 1 are given the data on normal somatic development for these 3 series of rats. The chief

TABLE 1A. WEIGHTS, IN GRAMS, OF 59 THYMECTOMIZED RATS (F_1 TO F_5) CONTRASTED WITH 82 OF THEIR UNOPERATED LITTER MATES. THYMECTOMY DOES NOT MATERIALLY AFFECT THE GROWTH OF THE INDIVIDUAL ANIMALS CONCERNED.

Age, Days	Thymectomized Animals	Unoperated Litter mates
10	12.7	12.7
20	24.2	24.1
30	41.7	42.1
40	61.8	62.5

difference to be found is in the earlier gonadal development observed in the rats of the Philadelphia Institute for Medical Research.

RESULTS OF THYMECTOMY IN SUCCESSIVE GENERATIONS OF RATS

Growth and Development of Test Rats of the First Generation (F_0). As a result of thymectomy in the original group of 8 rats, no abnormality in growth or behavior was noted. Likewise, irrespective of the generation concerned, thymectomy itself exercised no significant effect on the growth and development of the individual animals. This was evident in several instances in which only 2 or 3 animals of a large litter were subjected to operation. The growth and development of the operated animals did not differ materially from that of the unoperated members of the litter. Therefore, no differentiation has been made between the rate of growth of thymectomized and non-thymectomized offspring. The data herein presented refer to and include both the operated and non-operated animals in each generation. The period between gestations was 34.5 days as compared with 28.3 days in the operative controls and

TABLE 2. SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMECTOMY STRAINS OF RATS CONTRASTED WITH DEVELOPMENT OF CONTROL ANIMALS

	Ears open	Teeth erupted	Hair appeared	Eyes open	Testes descended	Vagina open
Operative Controls	2–3 (2.9)	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_1	3–4 (3.4)	8–11 (9.4)	13–18 (14.8)	14–18 (15.9)	24–35 (30.4)	38–53 (45.1)
F_2	2–4 (3.3)	8–11 (9.5)	12–16 (14.2)	15–18 (16.2)	24–36 (30.7)	42–61 (52)
F_3	3–4 (3.2)	7–10 (9.6)	12–15 (13.6)	15–18 (16.3)	26–33 (30.4)	41–52 (46.5)
F_4	3–4 (3.5)	7–12 (9.6)	12–17 (14.8)	16–19 (16.8)	28–32 (30)	45–52 (48.8)
F_5	3–4 (3.4)	8–11 (9.7)	13–17 (14.6)	16–19 (17.7)	30–35 (32.4)	48–54 (51.9)

* Figures in parentheses represent average values, in days.

37 days in the Philadelphia Institute series. The litters were large, averaged 8.3 animals per litter; the individual rats appeared strong, and averaged 4.8 gm. at birth. (See Table 1A).

Second Generation (F_1). A total of 191 rats were born in 23 litters to 4 pairs of thymectomized animals of the first generation (F_0). The litters varied

TABLE 3. AVERAGE WEIGHT, IN GRAMS, AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THE THYMECTOMY TEST STRAIN WITH PERCENTAGE UNDER THE WEIGHTS OF THE OPERATIVE CONTROLS

Age, days	Operative controls	F_1	% underweight	F_2	% underweight	F_3	% underweight	F_4	% underweight	F_5	% underweight
Birth	4.9	4.8	2	4.9	0	4.8	2	4.6	6	4.5	8
10	15.0	12.1	19	12.1	19	12.9	14	13.1	12	12.6	16
20	28.5	25.0	12	23.2	18	24.5	14	25.0	12	23.2	18
30	49.1	43.2	12	40.3	17	40.5	17	42.2	14	40.6	20
40	68.6	63.1	8	59.0	14	58.2	15	59.0	14	57.6	16
50	93.5	85.7	8	81.3	13	80.0	14	80.4	14	78.5	16
60	113.3	105.6	6	101.9	10	97.4	14	97.4	14	94.7	16
70	135.3	128.5	5	121.5	10	118.7	12	116.5	13	113.0	16

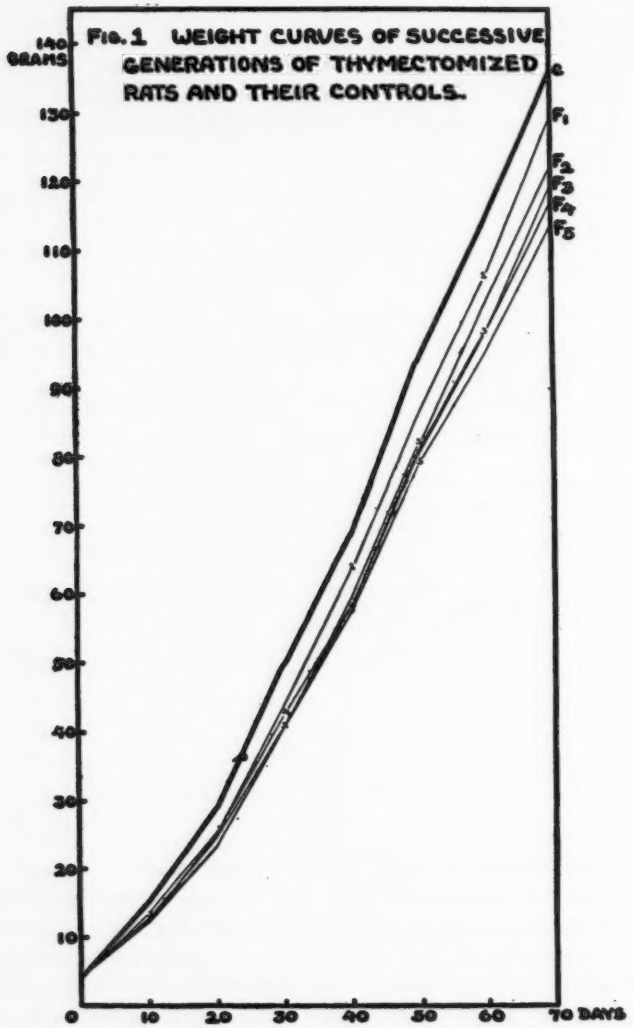
in size from 4 to 12, with an average of 8.3 animals per litter. Of these, 140 or 73 per cent survived the 70-day period of observation.

No uniform or marked retardation was noted in the opening of the ears, eruption of the teeth, development of the fur, opening of the eyes, descent of the testes, or opening of the vagina, although these somatic changes often appeared at the late limits of the normal range or just after it (table 2).

TABLE 4. AVERAGE LENGTH, IN CM., OF SUCCESSIVE GENERATIONS OF THYMECTOMY TEST STRAIN CONTRASTED WITH OPERATIVE CONTROLS

	20 days	30 days		20 days	30 days
Operative Controls	15.7	22.1	F_3	14.6	19.2
F_1	14.7	20.1	F_4	14.7	19.5
F_2	14.6	18.7	F_5	14.6	18.9

The important effect of thymectomy appeared in the rate of growth of the offspring reflected in their weight curves and body lengths. In figure 1 is shown the average growth curve of the entire group of 191 rats, constituting



the second generation (F_1). In table 3 is presented the average weights of this group at 10-day intervals. The retardation in growth was 2 per cent at birth, 19 per cent at 10 days, 12 per cent at 20 days, 12 per cent at 30 days, 8 per cent at 40 days, 8 per cent at 50 days, 6 per cent at 60 days, and 5 per cent at 70 days. The test animals of the second generation appeared definitely shorter and thinner than their controls between the ages of 20 to 30 days. In table 4 are

given the average body lengths measured from the tip of the nose to the tip of the tail, in a group of 20 control and 20 test animals. The test animals were 6.3 per cent shorter at 20 days and 9 per cent shorter than their controls at 30 days.

Third Generation (F_2). A total of 157 rats was born in 19 litters to 9 pairs of second generation (F_1) thymectomized rats. Of these 131 or 83 per cent survived the 70-day period of observation. As in the second generation no uniform or marked retardation in somatic development was noted, although these changes frequently occurred at or just after the late limits of the normal range.

The important changes resulting from thymectomy were noted in the rate of growth of the offspring as reflected in the weight curves, and in the measurements of body length. In figure 1 is shown the average growth curves of the entire group of 157 rats of the third generation (F_2) and in table 3 is presented the average weights of this group at 10-day intervals. The retardation in growth was 19 per cent at 10 days, 18 per cent at 20 days, 17 per cent at 30 days, 14 per cent at 40 days, 13 per cent at 50 days, 10 per cent at 60 days, and 10 per cent at 70 days. Reference to table 4 shows that a group of 20 of the test animals of the third generation were 7 per cent shorter at 20 days and 15.3 per cent shorter than their controls at 30 days.

Fourth Generation (F_3). A total of 166 rats was born in 21 litters in the fourth generation (F_3). The litters varied in size from 6 to 10 with an average of 7.9 animals per litter. Of the total, 145 or 87 per cent survived the 70-day period of observation. Again no uniform or marked retardation in somatic development was noted although in 9 litters the eyes opened on the 17th day and in one litter on the 18th day (table 2).

As in the preceding generations, there was noted a retardation in the rate of growth as reflected in the weight curves and body lengths of the offspring. In figure 1 is shown the average growth curve of the entire group of 166 rats, constituting the fourth generation, and in table 3 is presented the average weights of this group at 10-day intervals. The retardation in growth was 2 per cent at birth, 14 per cent at 10 days, 14 per cent at 20 days, 17 per cent at 30 days, 15 per cent at 40 days, 14 per cent at 50 days, 14 per cent at 60 days, and 12 per cent at 70 days. Although the average retardation in the fourth generation was not as marked during the first 30 days as it was in the third generation (F_2), it was evident that from 30 days on, this difference became greater until at 60 days it was 14 per cent less than the operative controls, as compared with the 10 per cent retardation of the third generation. The test animals appeared definitely shorter than their controls at 20 to 30 days. In table 4 are given the average body lengths of a group of 20 control and 20 test animals showing that the test animals were 7 per cent shorter at 20 days and 13 per cent shorter than their controls at 30 days.

Fifth Generation (F_4). A total of 201 rats was born in 26 litters of the fifth generation (F_4). The litters varied in size from 5 to 12 with an average of 7.7 animals per litter. Of the total of 201 rats, 153 or 76 per cent survived the 70-day period of observation.

Up to the fifth generation (F_4), no uniform or marked retardation in

TABLE 5. AVERAGE WEIGHT, IN GRAMS, AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THE MALE SPLIT-TEST STRAIN COMPARED WITH WEIGHTS OF CONTROLS

Age, days	Operative controls	F_1	F_2	F_3	F_4
Birth	4.9	4.9	4.9	4.7	4.7
10	15.0	14.9	14.1	14.7	14.7
20	28.5	28.8	28.2	28.5	29.9
30	49.1	48.9	49.0	47.9	50.9
40	68.6	69.8	68.4	65.8	70.1
50	93.5	94.2	90.9	94.7	91.1
60	113.3	113.8	111.7	113.4	112.2
70	135.3	134.4	130.3	135.0	131.0

somatic differentiation had been observed, although the changes often appeared at the late limits of the normal range or just after it. In 11 litters of the fifth generation the first upper incisor teeth erupted at 10 days and in 1 litter at 12 days as compared with 8 days, on the average, in the controls. The eyes opened in 7 litters at 18 days, and in 2 litters at 19 days (fig. 2) whereas the average in the controls was 15 days (table 2). Definite retardation in development was thus evident.

In figure 1 is shown the average growth curve of the entire group of 201 animals, constituting the fifth generation (F_4), and in table 3 is presented the average weights of this group at 10-day intervals. It can be seen that although the retardation in growth up to 30 days is similar to that shown by the rats in the earlier generations, at 60 and 70 days it is greater than that shown by the fourth generation (F_3). As is shown in table 4, the average body length of these test animals was shorter at 20 and 30 days than that of their controls.

Sixth Generation (F_5). A total of 142 rats was born in 19 litters of the sixth generation (F_5). Of these, 102 or 71 per cent had reached the age of 70 days on Feb. 1, 1937.

The test animals of the sixth generation showed a definite retardation in the rate of growth, as reflected by the average weight curve (fig. 1), and the average weights at 10-day intervals (table 3). During the first 30 days this retardation in growth closely resembled that seen in the earlier generations. At

TABLE 6. SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF MALE SPLIT-TEST STRAIN CONTRASTED WITH DEVELOPMENT OF CONTROL ANIMALS

	Ears open	Teeth erupt	Hair obscures genitalia	Eyes open	Testes descend	Vagina open
Operative Controls	2–3 (2.9)	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_1	3–4 (3.3)	7–11 (9.5)	13–16 (14.1)	14–17 (15.8)	28–32 (30.6)	41–52 (46.6)
F_2	3–4 (3.5)	8–10 (8.7)	13–15 (13.1)	15–17 (15.8)	25–33 (30)	42–56 (46.8)
F_3	3–4 (3.5)	8–10 (8.7)	13–15 (13.8)	15–17 (16.1)	25–30 (28.2)	42–52 (45.5)
F_4	3–4 (3.1)	8–10 (8.6)	13–15 (13.7)	14–17 (15.7)	27–32 (29.5)	39–50 (46.7)

* Figures in parentheses represent average values, in days.

both 60 and 70 days, however, this was 16 per cent as compared with 14 and 13 per cent respectively in the animals of the fourth generation (F_3), evidencing an accruing effect in successive generations.

Noteworthy changes were seen in the rate of somatic development (table 2). In 1 litter the teeth erupted on the 8th day, in 9 on the 9th day, in 7 on the 10th day, and in 1 each on the 11th and 12th days. Similarly, the eyes opened on the 16th day in only 1 litter, in 8 on the 17th day, in 7 on the 18th day, and in 3 on the 19th (fig. 3). The appearance of these developmental changes on the days indicated shows a striking delay in somatic differentiation.

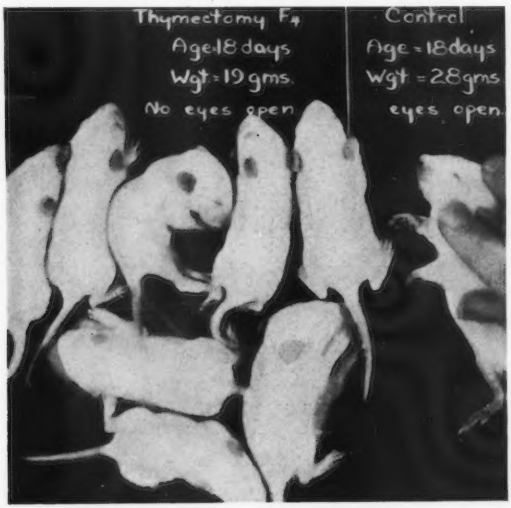


Fig. 2. Photograph of an entire litter of 8 fifth-generation (F_4) rats at the age of 18 days showing all eyes closed. Compare with operative control animal in which eyes opened on the 15th day.

EFFECTS OF THYMECTOMY IN SPLIT-TEST STRAIN

Methods. Six pairs of albino rats (Wistar strain) of known parentage were selected from the normal stock animals of the Philadelphia Institute for Medical Research. Three males were thymectomized, mated to 3 normal females; and 3 females were thymectomized and mated to normal males. The former group was designated the male split-test strain, and the latter the female split-test strain. To advance the male split-test strain to the next generation only one male in one litter of each pair of parent animals was thymectomized and mated to normal female litter-mates. Thus the offspring in each generation of the male split-test strain were young born to parent rats, the male of which had been subjected to thymectomy, and the female of which was a normal litter-mate. In the female split-test strain, thymectomized female rats were mated to normal male litter-mates; thus the offspring in the latter group were born to parent rats, the males of which were normal and the females of which were thymectomized litter-mates. Observations were made and data collected

in a manner identical with that already described for the thymectomy test strain.

Male Split-Test Strain (Male Parent Thymectomized, Female Parent not Operated). A total of 496 rats were studied, distributed as follows: second generation (F_1) 89, third generation (F_2) 128, fourth generation (F_3) 148, and fifth generation (F_4) 141. Reference to table 5 shows that there was essentially no difference between the average weights at 10-day intervals of the test animals in each of these 4 generations and their operative controls. Likewise no striking difference was seen in the opening of the ears, eruption of the teeth, appearance of hair, opening of the eyes, descent of the testes, and opening of the vagina (table 6).

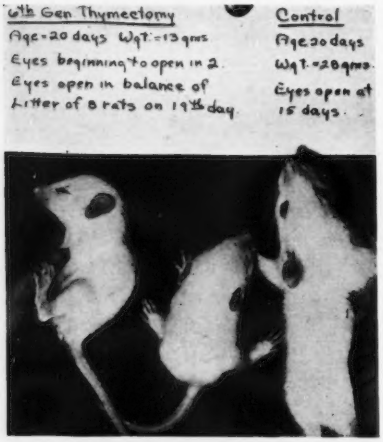


Fig. 3. Photograph of 6th generation (F_5) thymectomy test strain rats contrasted with operative control animal showing marked retardation in growth and development.

Female Split-Test Strain (Female Parent Thymectomized, Male Parent Not Operated). A total of 460 rats were studied, distributed as follows: second generation (F_1) 85, third generation (F_2) 143, fourth generation (F_3) 142, and fifth generation (F_4) 90. As with the male split-test strain, it was noted that there was essentially no difference between the average weights at 10-day intervals of the test animals in each of these 4 generations and their operative controls (table 7). Similarly, somatic development in the test strain showed no noteworthy deviation from that of the control animals (table 8).

DISCUSSION

This investigation was started Oct. 10, 1934, and at the time of this writing (Feb. 1, 1937) had continued for more than 27 months. It covers observations made on a total of 2,029 rats, distributed as follows: operative controls 208; thymectomy test strain, first generation (F_0) 8; second generation (F_1) 191; third generation (F_2) 157; fourth generation (F_3) 166; fifth generation (F_4) 201; sixth generation (F_5) 142; male split-test strain second (F_1) to fifth generation (F_4) 496; and female split-test strain second (F_1) to fifth generation (F_4), 460.

Thymectomy was performed at the average age of 21 days because then the thymus gland is well encapsulated and loosely adherent to neighborhood mediastinal tissues. In older rats the gland is more firmly attached and cannot be removed without frequently injuring important surrounding structures. In younger rats, the operator cannot be sure that the entire thymus gland has been removed. Finally, if the thymus functions as a gland, it probably does so prepubertally, hence it is important to remove it as early as is consistent with the best results.

TABLE 7. AVERAGE WEIGHT, IN GRAMS, AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THE FEMALE SPLIT-TEST STRAIN COMPARED WITH WEIGHTS OF CONTROLS

Age, days	Operative controls	F_1	F_2	F_3	F_4
Birth	4.9	4.6	4.6	4.9	4.7
10	15.0	14.9	14.3	15.3	15.8
20	28.5	28.8	28.7	29.1	29.3
30	49.1	49.9	48.1	50.7	50.2
40	68.6	68.0	68.3	69.6	70.0
50	98.5	93.2	93.0	98.3	97.2
60	113.3	111.8	110.2	113.0	110.2
70	135.3	133.0	130.0	135.2	130.8

Thymectomy in successive generations of parent rats has resulted in a definite retardation in the rate of growth and a moderate delay in somatic development. Of a total of 108 litters comprising the test rats of the second, third, fourth, fifth and sixth generations, one second generation litter consisting of 2 males showed a rate of growth greater than that of the operative controls. In the remaining 107 litters the rate of growth fell below the growth of the controls. This retardation in growth was most marked during the prepubertal period of life, reaching a maximum average retardation of 20 per cent at the age of 30 days in the sixth generation (F_5). At 70 days, however, each succeeding generation of the thymectomy test strain fell further below the weight of the controls than the preceding generation. In the thymus treated strain (4), the enhanced growth and development occurred during the early weeks of life, indicating an acceleration in attaining the prepubertal level of growth. In the thymectomy test strain, although retardation was most marked in the prepubertal period, the accruing retardation in growth was most apparent in the later weeks of prepubertal life. At the age of 120 days, the effects of thymectomy on growth and development were no longer apparent, the animals having attained normal weight.

The delay in differentiation following thymectomy is not as striking as the retardation in growth, particularly in the earlier generations. However, this delay in vital phenomena is better exemplified by the test rats of later generations, particularly the sixth (F_5). For example, in the thymectomized rats the upper incisors erupted, the eyes opened, the testes descended, and the vagina

TABLE 8. SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF FEMALE SPLIT-TEST STRAIN CONTRASTED WITH DEVELOPMENT OF CONTROL ANIMALS

	Ears open	Teeth erupt	Hair obscures genitalia	Eyes open	Testes descend	Vagina open
Operative Controls	2–3 (2.9)	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_1	2–4 (3)	8–11 (9.3)	13–16 (13.9)	14–17 (15.4)	28–36 (32.3)	40–61 (49.5)
F_2	2–4 (3)	8–9 (8.7)	13–14 (13.7)	13–16 (15.5)	25–31 (29)	40–49 (44.3)
F_3	3–4 (3)	7–10 (8.5)	13–15 (13.8)	14–16 (15.3)	26–31 (29.7)	41–48 (44.7)
F_4	2–3 (2.9)	7–9 (8)	11–15 (13.5)	14–16 (15.4)	28–31 (29.5)	45–54 (46.5)

* Figures in parentheses represent average values, in days.

opened at 9.7, 17.7, 32.4, and 51.9 days as compared with 8.5, 15.2, 30.2, and 44.3 days, respectively, in the operative controls.

Thymectomy in successive generations of males and females mated to normal litter mates is not effective in altering growth and somatic development in the offspring; both parents must be thymectomized in order to obtain results.

As an explanation of the retardation observed, a lack of milk supply on the part of the thymectomized mother seems inadequate, since this retardation persists in offspring nursed by normal foster mothers. The operation, per se, can be eliminated as a factor since in a number of instances in which the thymus gland had been removed from several of the animals of a large litter, the growth of the operated and non-operated members was practically identical. Again, no retardation in growth was noted in a large series of control animals whose parents had been subjected through 4 successive generations to the operative technique of thymectomy without actual removal of the gland. Finally, it is to be recalled that thymectomy was done at the average age of 21 days. Prior to this, the ears had opened, the teeth had erupted, hair had appeared, the eyes had opened, all somewhat later than normal, and the most pronounced retardation in growth had occurred during the first 3 weeks of life. One is therefore justified in concluding from these observations that the operation, the shock, hemorrhage and other factors incident thereto have played no significant rôle in the production of this delay.

Surgical thymectomy offers a better approach to the complete removal of the thymus gland than does the roentgen ray because the operator can speak with assurance about the completeness of the operation, whereas the roentgenologist can only surmise that with a certain intensity and duration of irradiation he has produced a complete involution of the gland with subsequent elimination of all of its active principle. Likewise, the roentgenologist cannot be sure that irradiation has been confined solely to the thymus gland. In a small rat, it is difficult to eliminate the effect of stray rays on neighboring glands; surgical thymectomy therefore, probably limits injury to the endocrine system more effectively than does irradiation.

There are two fundamental facts emerging from these studies. The effect of thymic deficiency is the antithesis of that obtained from an excess of thymus gland; this is seen especially in the contrast between the eyes opening at 19 days in thymus deficiency (fig. 3), and their opening at 3 days following thymus excess (4). The extent of retardation becomes amplified as thymectomy is carried through successive generations.[3]

SUMMARY

Thymectomy in successive generations of parent rats caused a retardation in growth and development in the offspring. This retardation is amplified as thymectomy is carried through succeeding generations to the 6th.

[3] Just as we felt that maximum acceleration in growth and development was attained in the 10th generation thymus-test strain, we feel that maximum retardation in growth and development occurred in the rats of the 6th generation (F_6) thymectomy test strain. Biological data now available on 5 litters of the 7th generation thymectomy rats indicates that the vital phenomena are not as markedly retarded as in the preceding generations.

Thymectomy in successive generations of males mated to normal female litter-mates did not affect the growth and development of the offspring, nor did thymectomy in successive generations of females mated to normal male litter-mates. Both parents must be thymectomized in order to produce retardation in the rate of growth and development of the offspring.

The results obtained in these studies seem to indicate that the thymus glands of the parents are concerned in growth and development of the offspring.

REFERENCES

1. Einhorn, N. H., and L. G. Rowntree: Endocrinology **20:** 342. 1936.
2. Einhorn, N. H., and L. G. Rowntree: Science **83:** 443. 1936.
3. Donaldson, H. H.: The Rat. 2nd ed. Wistar Institute of Anatomy and Biology, Philadelphia, 1924.
4. Rowntree, L. G., J. H. Clark and A. M. Hanson: J.A.M.A. **103:** 1425. 1934. Tr. A. Am. Physicians **49:** 252. 1934. Arch. Int. Med. **56:** 1. 1935.
   Rowntree, L. G., J. H. Clark, A. Steinberg and N. H. Einhorn: Tr. A. Am. Physicians **51:** 148. 1936.

STUDIES ON THE PHYSIOLOGY OF LACTATION. VII. LACTATION IN THYROIDECTOMIZED RATS AND GUINEA PIGS[1]

WARREN O. NELSON AND CHARLES E. TOBIN[2]
From the Departments of Anatomy, Wayne University and Yale University
DETROIT, MICHIGAN AND NEW HAVEN, CONNECTICUT

A considerable amount of progress has been made since the original studies on the lactogenic hormone of the anterior pituitary by Stricker and Greuter (1), Corner (2), and Nelson and Pfiffner (3, 4), toward an understanding of the physiology of milk secretion. These and other confirmatory studies were made on animals with intact hypophyses and thoroughly demonstrated the relation of the anterior lobe to the initiation of lactation. The ablation of the pituitary in lactating animals demonstrated the necessity of that gland for the maintenance of milk secretion. See Nelson (5) for a review of these studies.

It was reasonable to suppose that the administration of lactogenic hormone to hypophysectomized animals would result in the initiation of lactation. However, the results of such studies have been conflicting. Lyons, Chaikoff and Reichert (6) and McPhail (7, 8) reported the initiation of lactation in hypophysectomized dogs, ferrets, and cats while Selye, Collip and Thomson (9) were unable to do so in the rat. In the summer of 1935 during the preparation of a review on the control of lactation the senior author made a study of several controversial points relating to the mammary glands. Among these was the problem of lactation in hypophysectomized animals. It soon became apparent that lactation could be induced in the total absence of the hypophysis if crude pituitary extracts were used. However, if refined preparations of lactogenic hormone were employed it was not possible to initiate or maintain lactation. These results seemed to indicate that some hormonic factor in addition to the lactogenic hormone is involved in lactation. In collaboration with Dr. Robert Gaunt, who had shown that the disturbance of lactation which follows adrenalectomy could be corrected by the administration of adrenal cortical hormone (10), a study was made of the relation between the pituitary and the adrenals in lactation (11). In addition to further demonstrating that refined lactogenic hormone would not induce lactation in hypophysectomized guinea pigs it was shown that the simultaneous administration of the lactogenic and cortical hormones would initiate and maintain lactation. These findings have been confirmed by Gomez and Turner (12). Nelson and Gaunt (13, 14) have extended their original studies by showing that the simultaneous administration of the lactogenic and adrenotropic hormones would initiate lactation. In addition it was shown that lactogenic hormone would not induce lactation in adrenalectomized guinea pigs unless cortical hormone was also administered. These results have established a definite relation between

[1] Aided by grants from the Anna Fuller Fund and the Fluid Research Fund of Yale University.
[2] Osborne Zoological Laboratory, Yale University. Research assistant in the Department of Anatomy, Yale University during the summer of 1936.

the hypophysis and the adrenal cortex in the control of lactation. The significance of this relationship has been discussed by Nelson (15). However, the close relation of the thyroid to the pituitary in other physiological processes suggested that it, too, might be concerned in lactation. Weichert and Boyd (16) have noted that the administration of thyroid to pregnant rats resulted in a more rapid appearance of secretory products in the alveoli. Graham (17, 18) reported that thyroidectomy in the cow caused a decline in milk yield and milk fat, and that the administration of desiccated thyroid or thyroxin resulted in a rise in milk and milk fat.

These studies have indicated that the removal of the thyroid in lactating animals or the administration of thyroid with lactogenic hormone to hypophysectomized animals might be expected to give evidence of a relation of the thyroid to lactation. As one phase of this study we examined the effect of thyroidectomy upon a group of pregnant rats. As the results of a separate study on non-pregnant thyroidectomized rats had indicated that the animals of our colony regularly survived the removal of the thyroid and such parathyroid tissue as is regularly present within the rat thyroid, we took no precautions against parathyroid insufficiency. We were, therefore, surprised to note a high incidence of tetany in our pregnant females. Six animals succumbed and most of the others had one or more severe attacks. In a number of instances the animals were saved only through the administration of calcium salts by stomach tube.

TABLE 1. FEMALES THYROIDECTOMIZED DURING PREGNANCY

Rat No.	No. of young born	No. of living young	Average Weight of Young					No. of young at 28th da.
			1st day	7th day	14th day	21st day	28th day	
T1	8	8	5.1	13.2	17.5	24.7	42.4	7
T2	8	7	4.8	12.6	18.2	26.2	46.7	7
T3	7	6	4.9	14.8	21.7	27.5	43.8	6
T4	8	8	5.0	14.0	20.6	26.3	45.4	8
T6	10	10	4.7	12.2	17.9	22.8	40.6	9
T7	8	8	5.1	13.3	21.3	26.2	48.2	8
T9	7	7	5.0	13.8	20.8	25.9	43.4	7
Averages of series A		7.7	4.9	13.4	19.7	25.6	44.3	7.4
T12	8	6	5.2	14.6	21.0	27.2	48.5	6
T13	9	6	5.0	13.9	17.3	22.8	41.4	6
T14	8	6	4.9	12.3	16.8	22.5	42.7	6
T15	5	5	5.1	14.8	23.9	29.6	44.9	5
T17	9	6	4.8	12.7	18.7	23.4	43.1	5
T18	5	5	4.9	13.8	19.6	25.7	44.7	5
T19	6	6	5.3	15.1	23.4	30.3	46.3	6
T21	9	6	5.0	14.4	18.5	24.3	45.8	6
Averages of series B		5.8	5.0	13.9	19.9	25.7	44.7	5.6
Averages of 27 normal litters		7.5	5.1	13.7	20.3	25.2	45.2	7.1
Averages of 9 normal litters (reduced to 6)		6.0	5.0	14.1	20.1	26.1	45.4	5.9

Twenty-one rats were thyroidectomized between the 13th and 16th days of pregnancy. Of these, 15 survived through gestation and parturition. In all instances the mothers exhibited normal interest in their offspring and, as table 1 shows, lactation apparently was not affected by the removal of the thyroid. The litters in series A were not reduced while in series B they were, except in 2 instances where only 5 were born, reduced to 6 pups. Occasionally a pup died during the period of nursing, but this was no more frequent than in control litters. On the whole there was no evidence that thyroidectomy in any way influenced lactation.

All except 3 of the females used in the above study were sacrificed and the thyroid region removed for serial section. In only one instance (T 17)

TABLE 2. FEMALES THYROIDECTOMIZED BEFORE THE INCIDENCE OF PREGNANCY

Rat No.	Period of thyroidectomy	No. of young born	No. of living young	Average weight of young					No. of surviving young
				1st day	7th day	14th day	21st day	28th day	
T18	63	4	4	5.0	14.1	20.8	26.7	48.9	4
T18	128	5	4	4.9	15.2	20.7	28.2	47.3	4
T19	75	6	6	4.8	12.7	17.9	24.3	42.7	6
T19	137	5	4	4.9			Litter died		
T21	68	4	3	5.1	15.4	21.2	30.8	54.3	3
T21	139	5	5	4.8	14.9	20.3	27.7	48.8	4
T29	57	2	2	5.3	14.7	23.8	32.7	56.7	2
T29	115	5	4	5.0	12.6	22.2	30.3	54.3	3
T30	49	3	3	4.9	15.2	23.1	31.8	49.7	3
T30	120	8	6	4.8	12.5	20.3	25.7	44.3	6
T31	20	1	1	4.9	17.2	29.4	38.5	62.7	1
T31	86	2	2	5.1	16.7	27.3	34.3	60.3	2
T32	63	10	9	4.8	12.7	16.8	22.5	43.3	8
T33	32	5	5	4.8	13.6	18.3	25.3	46.2	5
T33	89	4	4	5.0	14.3	20.3	29.8	52.1	4
T31	24	2	2	5.1	15.5	24.8	36.7	60.2	2
T36	88	5	4	4.9	14.7	20.5	26.3	46.2	4
T38	33	3	3	5.1	13.9	17.8	23.6	43.5	3
Averages		4.4	4.0	4.9	14.5	21.5	29.1	50.6	3.8

was any thyroid tissue found. This animal had a small fragment present in the region of the upper pole of the right thyroid.

The 3 animals allowed to survive and 8 other non-pregnant rats which had been thyroidectomized were mated with normal males. The vaginal smears of these animals were followed daily. It was observed that the estrous cycles of these animals were definitely disturbed, there being a tendency toward extended diestrous periods. Non-fertile copulations were more frequent in these animals than we have observed in control rats. However, in 10 of these animals at least one pregnancy was obtained. None of these animals died during their first pregnancy but 2 died in tetany during their second gestation. Table 2 shows the data collected on the litters of these females. It will be noted that although there is a tendency toward small litters, lactation, as judged by the growth of the young rats, seems to have been unaffected. In one instance, T 19, the litter died. This, of course, occurs occasionally in normal animals. As in

the first group the thyroid regions have been sectioned serially. No residual tissue was found.

Six female guinea pigs were thyroidectomized during the last 10 days of pregnancy. We have observed no instances of tetany in the guinea pigs of this or the following series. Inasmuch as young guinea pigs ingest solid food within a day or two after birth we have not made a study of the weights of the young born to our operated females. However, it was observed that lactation was initiated and continued in a perfectly normal fashion in all of these animals.

Four male and 2 young female guinea pigs were injected with 40 R.U. of theelin[3] daily for 28 days. As we have repeatedly shown (19), such animals will invariably lactate spontaneously within a few days after the cessation of estrin injections providing the pituitary is not removed (20). These animals were thyroidectomized and 2 days later the administration of theelin was stopped. In each instance lactation began within 65 hours and continued for at least 10 days, the usual lactation period in the guinea pig.

Two male guinea pigs were thyroidectomized and injections of theelin (30 R.U. daily) were given for 28 days. Lactation occurred in these animals after the cessation of theelin treatment. These experiments demonstrate that mammary development and lactation can occur in the complete absence of the thyroid gland. Nelson and Hickman, in experiments soon to be published, have observed complete proliferation of the mammary gland in thyroidectomized rats which had been injected with estrin.

Although the above experiments had demonstrated that the absence of the thyroid gland does not interfere seriously with lactation, it was of interest to investigate the possibility that the administration of thyroid hormone would be of value in the initiation of lactation in suitably prepared animals. In this regard to seemed of value to utilize the procedure used by Nelson and Gaunt in studying the effect of the adrenal cortical hormone in hypophysectomized guinea pigs receiving lactogenic hormone. Thus we prepared a series of male and female guinea pigs (400 to 450 gm. in weight), injecting them with 30 R.U. of theelin daily for 30 days. They were hypophysectomized and theelin injections were discontinued. Injections of lactogenic hormone[3] (10 mg. daily) were begun the day after hypophysectomy. As has been shown by Nelson and Gaunt this treatment does not initiate lactation unless cortical hormone, adrenotropic hormone, or large amounts of salt solution are also administered. Instead of these we gave the animals in this series 0.3 to 0.5 grams of desiccated thyroid daily. This was given by stomach tube with 5 gm. of sugar in 2 doses each day. As Nelson (20) has reported, it is important to supply hypophysectomized guinea pigs with sugar at regular intervals during the first week after operation.

Seven of the guinea pigs in this series lived at least 10 days. In no instance did lactation occur during the period of treatment with lactogenic hormone

[3] The theelin, lactogenic hormone, and desiccated thyroid were generously supplied supplied by Dr. Oliver Kamm of Parke, Davis & Co.

and thyroid (8 to 15 days). In 2 animals cortical hormone was substituted for thyroid after the 8th day. Both of these animals lactated on the third day of such treatment. Hypophysectomy, as shown by microscopic examination of the sellae, was complete in these animals.

Two parturient females which were lactating, and one male which had been induced to lactate, were hypophysectomized and given lactogenic hormone plus the desiccated thyroid. Lactation stopped in these animals within 2 days. This is the usual result of hypophysectomy in lactating guinea pigs (20), even in those receiving lactogenic hormone, and indicates that the thyroid hormone had failed to influence the results that normally would have followed the removal of the hypophysis. Here as in our other experiments in which thyroid was given we had no conclusive evidence that the ingested thyroid had exerted any physiological activity. The weight loss which invariably follows hypophysectomy was of the usual order in these animals and the percentage of survivals was about that of any group of hypophysectomized guinea pigs. However, in view of the many examples, both experimental and clinical, of the effectiveness of orally administered thyroid we believe that it is reasonably safe to assume that the thyroid supplied to our animals was physiologically active.

DISCUSSION

These experiments show that mammary development and normal lactation can occur in rats and guinea pigs in the absence of the thyroid gland. Insofar as we have been able to detect, lactation in our experimental animals has been entirely normal in amount and duration. As we have made no tests of the milk constituents, no statement concerning the quality of the milk can be made other than that it probably did not vary far from the normal since the young animals showed no evidence of nutritional deficiencies. In accord with the results on thyroidectomized animals was our demonstration that the thyroid hormone, as represented by desiccated gland substance, does not initiate lactation when given with the lactogenic hormone to hypophysectomized guinea pigs.

However, as Nelson and Tobin (21) stated in a preliminary report of this work, the demonstration that lactation may occur in thyroidectomized animals should not be interpreted as indicating that the thyroid has no influence on normal lactation. It is reasonable to suppose that a substance which so profoundly influences metabolic activity should play some rôle in a physiological activity as complex as milk secretion and its control appears, in the light of recent evidence, to be. The major controlling factor in lactation is, as has been repeatedly shown, the lactogenic hormone of the pituitary. This appears to be a specific action and seems to be directly upon the cells of the mammary alveoli. Of only slightly less importance is the adrenal cortex (11, 14). Whether the activity of the adrenal cortex is directed through the influence of the cortical hormone on salt and water metabolism, as some of Nelson and Gaunt's results would indicate, or through some less understood function, is not entirely clear. It is hoped that studies in progress will furnish

a clue to this problem. Through its relation to the adrenals the adrenotrotropic hormone of the hypophysis, which would appear to be separate from the lactogenic factor, is related to lactation. In addition, it is likely that the factors controlling carbohydrate metabolism play an important rôle in milk secretion. Here, too, it is supposed that an anterior lobe hormone is an important factor, but its identity as a separate hormone has not been proven.

The small size of the litters delivered by the female rats which had been thyroidectomized prior to the incidence of pregnancy reflects the occurrence of distinct disturbances in the reproductive rhythm following removal of the thyroid. We have observed marked prolongations of the estrous cycle, the frequent occurrence of infertile copulations, and, of course, the tendency to bear small litters. The disturbances associated with reproduction may be due to the marked changes which occur in the anterior pituitary after thyroidectomy. The basophiles increase in number and size, and, to a certain degree, react in a manner similar to their response to gonadectomy (22). We have observed these changes in the pituitaries of our animals, and although we cannot consider the question at this time, have considered them as related to the disturbances of reproduction.

SUMMARY

Fifteen rats thyroidectomized during the last half of pregnancy continued to term, delivered normal litters, and suckled them successfully. Ten rats thyroidectomized prior to the incidence of pregnancy delivered and suckled successfully one or more litters, 17 in all. Six female guinea pigs were thyroidectomized during the last 10 days of pregnancy. In each instance parturition and lactation occurred in a normal fashion.

Four male and two female guinea pigs were injected with estrin for 28 days. In each instance the thyroid was removed and the injections of estrin withdrawn. All animals began lactating within 3 days. Two male guinea pigs were thyroidectomized and injected with estrin for 28 days. In each instance lactation occurred following the cessation of estrin treatment.

Seven guinea pigs which had received injections of estrin for 30 days were hypophysectomized. Treatment with lactogenic hormone and orally administered desiccated thyroid failed to initiate lactation. Two of these animals were subsequently induced to lactate by substituting cortical hormone for the thyroid substance. Three lactating guinea pigs were hypophysectomized and given lactogenic hormone and thyroid substance. Lactation ceased within 2 days.

This evidence shows that mammary gland development and lactation may occur in the absence of the thyroid gland. Furthermore, the thyroid hormone when given with lactogenic hormone failed to initiate or maintain lactation in hypophysectomized animals.

REFERENCES

1. Stricker, P., and F. Greuter: Presse méd. **37:** 1268. 1929.
2. Corner, G. W.: Am. J. Physiol. **95:** 43. 1930.
3. Nelson, W. O., and J. J. Pfiffner: Proc. Soc. Exper. Biol. & Med. **28:** 1. 1930.
4. Nelson, W. O., and J. J. Pfiffner: Anat. Rec. **51:** 51. 1931.
5. Nelson, W. O.: Physiol. Rev. **16:** 488. 1936.

6. Lyons, W. R., I. L. Chaikoff and F. L. Reichert: Proc. Soc. Exper. Biol. & Med. **31:** 303. 1933.
7. McPhail, M. K.: Proc. Roy. Soc., London, s. B. **117:** 34. 1935.
8. McPhail, M. K.: Proc. Roy. Soc., London, s. B. **117:** 45. 1935.
9. Selye, H., J. B. Collip and D. L. Thomson: Endocrinology **18:** 237. 1934.
10. Gaunt, R., and C. E. Tobin: Am. J. Physiol. **115:** 558. 1936.
11. Nelson, W. O., and R. Gaunt: Proc. Soc. Exper. Biol. & Med. **34:** 671. 1936.
12. Gomez, E. T., and C. W. Turner: Proc. Soc. Exper. Biol. & Med. **35:** 365. 1936.
13. Gaunt, R., and W. O. Nelson: Anat. Rec. **67:** 18. 1937. (Suppl.).
14. Nelson, W. O., and R. Gaunt: Proc. Soc. Exper. Biol. & Med. **36:** 136. 1937.
15. Nelson, W. O.: Am. J. Anat. **60:** 341. 1937.
16. Weichert, C. K., and R. W. Boyd: Anat. Rec. **59:** 157. 1934.
17. Graham, W. R.: J. Nutrition **7:** 407. 1934.
18. Graham, W. R.: Biochem. J. **28:** 1368. 1934.
19. Nelson, W. O.: Endocrinology **19:** 187. 1935.
20. Nelson, W. O. Proc. Soc. Exper. Biol. & Med. **33:** 222. 1935.
21. Nelson, W. O., and C. E. Tobin: Anat. Rec. **67:** 110. 1936. (Suppl.).
22. Severinghaus, A. E., G. K. Smelser and H. M. Clark: Proc. Soc. Exper. Biol. & Med. **31:** 1127. 1934.

A COMPARISON OF THE KETOSIS DEVELOPED DURING FASTING BY OBESE PATIENTS AND NORMAL SUBJECTS

EATON M. MacKAY AND JAMES W. SHERRILL
From the Scripps Metabolic Clinic
LA JOLLA, CALIFORNIA

The general cause of obesity is obviously the ingestion and utilization by an individual of calories in excess of those expended by the organism (1). But obesity is after all only a symptom and there is every reason to suppose that it may result from numerous causes, for it would be most unreasonable to assume that all types of obesity are alike. However, our present attempts to classify obese patients are based not on truly objective findings but rather on the broad and more or less uncertain grounds of clinical observation. A possible approach to the classification of obesity appeared to be through a study of the extent of the ketosis developed during fasting in these patients. The route of the normal oxidation of fat in the body is not known with certainty, but is generally assumed to be through such a route that the so called 'ketone bodies'—beta hydroxybutyric acid, acetoacetic acid and acetone—are produced enroute to oxidation to carbon dioxide and water. Under conditions in which either the absolute amount of carbohydrate available is insufficient or there is a relative lack of 'oxidizable carbohydrate' this oxidation is not complete, the ketone bodies appear in the blood and urine, and we have a ketosis. The extent of this ketosis is apparently always dependent upon the rate of oxidation of carbohydrate in all of its forms (preformed and from protein, glycerol of fat and possibly from other sources) in relation to the amount of fat which is being burned. In the fasting subject, after the first couple of days, the liver glycogen becomes exhausted and the organism is solely dependent upon stored fat and stored and active tissue protein for its source of heat and energy. *A priori,* it might be supposed that obese patients would develop a greater fasting ketosis than non-obese subjects because of the nature of their metabolic mixture (oxidizable carbohydrate vs. fat) due to the excessive fat deposits which are presumably available for oxidation. If stored fat varied in its availability this difference in the nature of obesity might be determined by the extent of the ketosis developed.

The belief is generally held that in obesity the onset or severity of the ketosis of fasting is no different from that in non-obese individuals. If there were no direct evidence on the subject most experts in metabolic physiology would probably still hold the view that Folin suggested as being reasonable in the absence of experimental data more than 20 years ago (2), namely that "Since fat persons tend to store fat more readily than lean ones, they

Table 1.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Subject	*R.M.*	*M.G.*	*G.C.D.*	*V.T.*	*R.B.*	*10439*	*10317*	*10371*	*Mrs. B.*	*Mrs. M.*	*J.S.B.*	*P.W.S.*	*P.L.*	*H.J.D.*	*10172*	*C.T.*
Source Ref.	(3)	(3)	(3)	(3)	(3)				(2)	(2)	(3)	(3)	(3)	(3)		(3)
Sex	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♂	♂	♂	♂	♂	♂
Age						18	51	22	44	48					22	
No. of Fasts	1	1	3	3	3	1	2	1	2	2	4	4	3	6	2	2
Inches Height	66	57	62	64	64	63.2	64.5	64.5	64.5	49	70.5	68	68	70.5	74.4	70
Body Weight																
Observed, lb.	124	100	135	146	156	161	269	241	391	238	154	143	152	192	287	240
Ideal, lb.	131	100	121	126	127	121	146	127	133	70	156	146	146	160	193[1]	154
Obesity Index %[4]	−5	0	12	16	22	25	84	90	199	240	−1	−2	4	20	49	56
Body Surface																
Observed sq. m.	1.63	1.35	1.62	1.70	1.76	1.75	2.19	2.10	4.50	1.71	1.88	1.77	1.82	2.06	2.51	2.26
Ideal sq. m.			1.53	1.59	1.60	1.55	1.69	1.60	1.60	1.03			1.78	1.88	2.13	1.86
Ketone body excretion in gm. per sq. m. per day; body surface calculated from observed weight.																
1	0.34	2.17	0.03	0.99	0.12	0.73	0.04	0.03	0.00	0.09	0.04	0.02	0.03	0.09	0.85	0.02
2	4.03	4.47	2.24	4.62	5.67	2.97	0.10	0.07	0.00	1.29	0.75	0.71	0.32	1.77	1.36	0.25
3	6.11	4.96	10.51	7.40	9.93[3]	5.58	0.13	0.37	0.21	6.12	2.32	1.47	0.86	3.42	4.14	0.63
4	6.24	7.30	7.57[2]	5.53	8.18[3]	6.13	0.44	0.23	0.95	8.44	3.82	2.16	1.45	4.01	5.31	2.05
5						5.76	1.23	1.26	2.22[2]	8.85[2]					6.96[2]	
Ketone body excretion in gm. per sq. m. per day; body surface calculated from ideal weight.																
1	0.34	2.17	0.03	1.06	0.13	0.83	0.05	0.04	0.00	0.14	0.04	0.02	0.03	0.08	1.00	0.02
2	4.03	4.47	2.37	4.93	6.23	3.35	0.13	0.09	0.00	2.25	0.75	0.71	0.30	1.94	1.60	0.30
3	6.11	4.96	11.15	7.92	10.91[3]	6.32	0.17	0.46	0.57	10.15	2.32	1.47	0.88	3.75	4.87	0.77
4	6.24	7.30	8.02[2]	5.92	9.02[2]	6.90	0.56	0.29	2.67	13.99	3.82	2.16	1.48	4.39	6.26	2.49
5						6.51	1.59	1.56	6.18[2]	14.69					8.20	
N excretion in gm. per sq. m. per day; body surface calculated from ideal weight.																
1	4.89	7.74	4.15	5.13	3.25	4.71	4.20	4.01	4.48	4.81	5.31	5.12		5.52	5.60	7.20
2	7.51	8.63	4.50	7.00	5.66	5.18	3.64	2.84	4.87	4.81	5.39	6.25		5.74	4.94	7.40
3	7.09	7.28	6.00	6.44	4.50[2]	5.03	3.52	2.23	4.73	6.31	6.46	6.91		6.59	4.94	7.67
4				5.53	6.68[2]	5.28	3.59	3.69	4.59	7.22	6.51	6.56		6.30	4.36	8.09
5						5.46	3.91	3.94	5.19[2]	5.04					4.25[2]	

[1] 10% added to regular standard weight because of the large frame of this individual.

[2] Average of 1 fast only.

[3] Average of 2 fasts only.

[4] Obesity Index $= \frac{\text{observed wt.—ideal wt.}}{\text{ideal wt.}} \times 100.$

probably tend to utilize it less readily, and that they therefore should be more subject to acidosis when compelled to live on their own tissue materials." As a result of an experimental investigation Folin and Denis (2) came to the conclusion that "Obesity is not a predisposing or contributing factor in the onset of the acidosis of starvation." If we examine their data we find that 2 obese subjects were studied. One of these developed an unusually high degree of ketonuria. In the other and more obese patient the ketonuria developed during fasting was not unusual. Deuel and Gulick (3) have also recently concluded that "Obesity is not a predisposing cause of acidosis." A critical examination of their data along with that of Folin and Denis together with some experiments of our own lead us to somewhat different conclusions.

The data of Folin and Denis (2), Deuel and Gulick (3) and our own are summarized in table 1. Our analytical methods were the same as those used by Deuel and Gulick (3). Observations were made over a fasting period of 4 or 5 days. In order to evaluate the degree of overweight in these patients we have used an 'obesity index' which is the excess weight of the observed weight over the ideal weight as a percentage of the latter. Although results presented on the basis of the body surface calculated from the observed weight show the points we wish to bring out here and are given in this manner in table 1, we prefer to use the body surface calculated from the ideal weight, whenever the subject is overweight, as a basis upon which to present our data. This is upon the reasonable assumption that the excess fat deposits in obesity are relatively inactive tissue. This view gains considerable support from measurements of the basal metabolic rate in obese individuals and the nitrogen excretion in fasting obese subjects must be referred to the body surface calculated from the ideal body weight, for, if shown in relation to the body surface calculated from the observed weight, it will often be ridiculously low. Consequently our discussion is concerned with the ideal weight body surface data.

A perusal of the data on male subjects shows that during fasting males who are simply fat, 14 and 15, developed a greater ketosis than those of normal weight, 11, 12 and 13. The most obese male subject, 16, had a very small ketosis, less in fact, than two of the normals. Although we did not make the observations upon this subject he is known to one of us, and clinically because of the extent and distribution of his fat he could not be classified as a case of simple obesity.

Three of the obese female patients, 7, 8 and 9, had a less marked fasting ketosis than would be expected in the normal subject. Two of these, 7 and 8, had not been naturally obese but gathered their weight quickly following evidence of ovarian deficiency. The other obese female patient, 9, with a small ketosis was not in our hands but her history indicates (2) that she had had an abdominal operation. At any rate it is not possible to say definitely that she was a case of simple obesity. The rest of the obese females, apparently suffering from 'simple obesity' developed a fasting ketosis as great or greater than we would expect in non-obese persons.

We conclude from the data presented here that unless an obese subject has a fasting ketosis a great deal lower than the average normal person he will probably develop a higher degree of ketosis than the non-obese individual. Obese patients who have less of a fasting ketosis than normals are probably suffering from a different type of obesity possibly related to disturbances in the endocrine system. This failure to develop a ketosis brings up the question of 'locked fat' in connection with the fat deposits in certain cases of obesity. Because of the ketogenic activity of certain anterior pituitary extracts (4) and their ability to move fat from the body stores to the liver (5) the functional state of this gland in obesity must be considered. Further studies of the extent of fasting ketosis as a possible means of classifying types of obese patients and perhaps gaining some insight into the mechanism of obesity are indicated. A short fast is a useful therapeutic agent for many obese patients and under the proper conditions is not difficult to carry out. Our patients, as well as the other subjects from whom the data used here were obtained, did not have their diet carefully controlled previous to fasting. There is considerable evidence that in further work of this kind rigid control of the preceding diet would be very desirable. Comparisons must of course be limited to groups of a single sex (3).

SUMMARY

New experiments along with data from the literature are used for a comparison of the extent of fasting ketosis in obese and non-obese subjects, which have hitherto been believed to be similar. Patients who were apparently only overweight and might be said to have simple obesity always developed as large and usually a greater ketosis than non-obese controls. A number of obese patients had a fasting ketosis much smaller in extent than is ever seen in a well nourished normal individual. Their examination suggested some endocrine disturbance in every case. The results suggest that the method is worthy of further use as a possible means of classifying obesity upon a physiological basis.

REFERENCES

1. Newburgh, L. H., and M. W. Johnston: J. Clin. Investigation **8:** 197. 1929.
2. Folin, O., and W. Denis: J. Biol. Chem. **21:** 183. 1915.
3. Deuel, H. J. Jr., and M. Gulick: J. Biol. Chem. **96:** 25. 1932.
4. MacKay, E. M., and R. H. Barnes: Am. J. Physiol. **118:** 184. 1937.
5. Best, C. H., and J. Campbell: J. Physiol. **86:** 191. 1936.

CLINICAL AND LABORATORY NOTES

STUDIES ON THE FUNCTION OF THE PINEAL GLAND

NELSON J. WADE*

In spite of repeated investigation the function of the pineal is still uncertain. Experiments involving ablation of the gland and the administration of preparations of the pineal have given conflicting results. Engel in a series of recent publications has advanced the opinion that preparations of the pineal inhibit the action of the gonadotropic hormones. According to these reports alkaline extracts of from ¼ to ½ of a human gland inhibit the action of 10 R.U. of gonadotropic hormone. Active preparations were secured from several species in the following amounts: human, 20-60; calf or beef, 30-40; hog, 10-15; and fowl, 5-7 R.U. of antigonadotropic hormone.[1, 2, 3, 4, 5] The unit was defined as one-tenth of the amount required to inhibit the action of 10 R.U. of gonad-stimulating hormone in infantile female rats. These preparations inhibited both the follicle-stimulating and the luteinizing reaction although the inhibition of the latter was more pronounced. The gonadotropic hormones were prepared from pregnancy urine and pregnant mare serum.[1,2,3]

In contrast to this, several other investigations have failed to yield similar results. Fleischmann and Goldhammer were unable to confirm these findings even with Engel's pineal extracts.[6] Vinals reported that macerated pineal tissue augmented the action of pregnancy urine in rabbits,[7] and Rowntree et al indicated that extracts from the pineal may cause precocious sexual development in rats after treatment for several generations.[8]

The reported effects of pineal preparations on estrus is likewise conflicting. Silberstein and Engel have reported that implants[9] or extracts[10] of ⅛ to ¼ of a human pineal or similar extracts prepared from beef glands[3] will cause estrus in spayed mice. These results were confirmed by Saphir[11] with implants of human pineals in mice, but not by Tarkham[12] using implants of calf pineals.

The data presented in this paper were obtained in a study of the antigonadotropic and estrogenic potencies of beef pineal glands. The pineal preparations used were: *1)* fresh beef pineal glands secured from the killing floor of the packing house,† macerated and injected as soon as possible; *2)* 0.5 per cent Na_2CO_3 extracts (PE 1-6) of acetone-dried beef pineals made according to the method of Engel. In some

* From the Department of Biology, Saint Louis University, St. Louis, Missouri.

† The author is indebted to Mr. A. G. Ackermann of the Laclede Packing Company for the glands used in these experiments.

[1] Engel, P.: Klin. Wchnschr. **13**: 266. 1934.
[2] Engel, P.: Ztschr. f. d. ges. exper. Med. **96**: 328. 1935.
[3] Engel, P.: Wien. klin. Wchnschr. **48**: 1160. 1935.
[4] Engel, P.: Ztschr. f. d. ges. exper. Med. **95**: 441. 1935.
[5] Engel, P.: Wien. klin. Wchnschr. **48**: 481. 1935.
[6] Fleischmann, W., and H. Goldhammer: Klin. Wchnschr. **15**: 1047. 1936.
[7] Vinals, E.: Compt. rend. Soc. de biol. **119**: 259. 1935.
[8] Rowntree, L. G., J. H. Clark, A. Steinberg, A. M. Hanson, N. H. Einhorn, and W. A. Shannon: Ann. Int. Med. **9**: 359. 1935.
[9] Silberstein, F., and P. Engel: Klin. Wchnschr. **12**: 908. 1933.
[10] Engel, P.: Ztschr. f. d. ges. exper. Med. **93**: 69. 1933.
[11] Saphir, W.: Endocrinology **18**: 625. 1934.
[12] Tarkham, A. A.: Endokrinologie, **18**: 234. 1937.

cases NaOH was used at a pH of 8.5; *3)* a suspension (PS) of finely ground acetone-dried pineal glands in distilled water or dilute alkali.

The gonadotropic preparations used were: *1)* pregnancy urine extracts (PU); *2)* an extract (AP) prepared from the anterior lobe of sheep pituitaries; *3)* an extract (MS) prepared from pregnant mare serum.

These preparations were administered to female rats 21 to 25 days old in 6 injections over a period of 48 hours. The animals were killed at the end of 100 hours, the ovaries weighed and then fixed in Bouin's fluid for later microscopic study. In some experiments the injection of the pineal preparations preceded the

TABLE 1. EFFECT OF PINEAL PREPARATIONS ON GONADOTROPIC ACTIVITY

Gonadotropic hormone	Method of administration	Pineal preparations	Method of administration	Equal to fresh pineals	No. of animals	Average ovarian weight
PU	Sub. dors.	PE 3	Sub. dors.	3–4	3	21.7
"	" "	—	—	—	3	20.0
"	" "	PE 3	Sub. dors.	3–4	3	22.5
"	" "	—	—	—	3	17.9
AP (6)[1]	" "	PE 3–6	Sub. dors.	3–4	21	33.3
"	" "	—	—	—	13	21.6
AP (9–12)	" "	PS	Sub. dors.	4	9	59.2
"	" "	—	—	—	6	26.0
AP (6)	" "	Fresh	Sub. dors.	1–2	4	27.2
"	" "	—	—	—	4	27.1
AP (12)	Intraper.	PS	Sub. dors.	4	7	21.2
"	"	—	—	—	4	19.2
"	Sub. vent.	PS	Sub. dors.	4	6	29.8
"	" "	—	—	—	3	25.4
"	Intraper.	PS	Intraper.	4	6	24.8
"	"	—	—	—	3	14.5
AP (15)	Sub. vent.	Fresh	Sub. dors.	2	10	23.0
"	" "	—	—	—	8	25.7
MS	" "	Fresh	Sub. dors.	2	8	15.4
"	" "	—	—	—	4	15.9

[1] Milligrams administered.

injection of the gonad-stimulating hormone up to 48 hours. Tests for estrogenic activity were performed in spayed rats and mice.

The pineal and gonadotropic preparations used, the method of administration and the results are shown in table 1. These results do not support the view that the pineal gland contains a substance antagonistic to the gonadotropic hormones. Instead, the results show a tendency for the pineal preparations to augment the action of gonad-stimulating hormones. Since the injections of these preparations were usually accompanied with varying degrees of inflammation and swelling, it was thought that the augmentation might be due to an altered process of absorption when both preparations were injected in the same region. When the sites of the injections were carefully separated, the increase in ovarian weight was slight, indicating that the reaction was not a specific effect of the pineal.

Sections from these ovaries show that follicle stimulation and luteinization were as marked in the animals receiving the pineal preparations as in those receiving only the gonadotropic hormone.

Since the ovarian weights of Engel's test animals were not given, and the rat unit is an unreliable basis of comparison, it is difficult to compare the degree of gonad stimulation in the two cases. The amount of gonad-stimulating hormone, however, was kept low so it is unlikely that the dosages used masked any antigonadotropic activity of the pineal preparations. The amount of pineal material, on the other hand, was considerably greater than that employed by Engel. In some cases the injection of the pineal preparations preceded that of the gonadotropic hormone by as

TABLE 2. ESTROGENIC ACTIVITY OF PINEAL GLANDS

Animals	No. of animals	Pineal preparations	Equal to fresh pineals	Days injected	Vaginal response	Days examined
Rats	4	PE 1–2	4	2	—	3– 4
"	2	PE 3	9	2	—	3– 4
"	2	Fresh	9.5	2	—	3– 8
"	4	"	6	3	—	6–10
Mice	2	PS	4	3	—	7–10

much as 48 hours without altering the response.

In making the tests for the presence of estrogenic material adult spayed rats and mice were used as shown in table 2. No estrogenic activity was observed in any of these tests. Since cornification may be produced by smearing alone[13] the vaginal smears were made only at the time estrogenic activity would be expected and then but once a day when the observations were extended over several days. The dosage used was so high in relation to the reported activity of these preparations that it would seem impossible to miss any activity if it were present. Rats were usually used in these tests since they were much better able to withstand the effects of the injections. These preparations were quite toxic for the mice and a number died from the effects. From these experiments it is felt that there was no estrogenic activity in the amount of pineal material administered.

Summary. The injection of preparations from 1 to 4 beef pineal glands failed to reveal antigonadotropic activity in infantile female rats and the injection of preparation from 4 to 9.5 beef pineals failed to reveal estrogenic activity in adult spayed rats.

[13] Wade, N. J., and E. A. Doisy: Proc. Soc. Exper. Biol. & Med. 32: 707. 1935.

STUDY OF THE GILFILLEN-GREGG SKIN TEST FOR PREGNANCY*

SAIM SAGLIK AND ELEMER SCIPIADES, JR.

The present study was stimulated on the one hand by the appearance of Gilfillen and Gregg's paper describing a skin test for the detection of pregnancy, and on the other hand by Hamlett's discovery that in the monkey prolan appears in the urine for a very limited period of time, namely from the 19th to the 25th day of pregnancy. It seemed desirable to extend the study to the monkey as well as other mammals for comparison with further control observations on women.

The theory underlying the Gilfillen-Gregg test is that the pregnant woman, having an abundant supply of prolan in the blood, should be immune to further injections of urinary prolan, whereas the non-pregnant woman should be prolan-sensitive. Their technique is as follows: Through a 26-ga. needle 2 minims of antuitrin-S is injected intradermally on the flexor surface of the forearm. Results are read a half-hour after injection. An area of erythema 25 to 30 mm. in diameter about the site of injection is considered a positive reaction and reflects the non-pregnant condition. Conversely, absence of the reaction within a half-hour is considered a positive test for pregnancy. It is claimed that the test is as reliable as that of Aschheim-Zondek, with the obvious advantages of simplicity and economy of time—an office procedure in fact.

Attention may here be called also to a paper by Dowel who in 1933 proposed using anterior pituitary extracts in a similar way; he gave no experimental data.

This Gilfillen-Gregg reaction is, however, not new. Porges and Pollatschek first suggested the idea to the Vienna Medical Society in March 1929 and claimed for the method accuracy and dependability, using the same technique as Gilfillen and Gregg. The Vienna workers, however, a few months later, after further trials, reported 18 per cent failures. Deutch, in November 1929, described the method as absolutely unreliable on the basis of injections of 45 pregnant and 65 non-pregnant women. Strauss in this country, using the test on 184 women, pronounced it too unreliable for use in general practice. Finally, while our own tests on animals were in progress, Weisman and Yerburry reported tests on 17 non-pregnant, 31 pregnant women, and 26 men. Their results showed the test to be much too unreliable to supplant the A-Z or the Friedman tests for pregnancy.

Experiments on Rodents. Two non-pregnant female rats, and 2 in the 8th day of pregnancy were injected intradermally on the right side of the abdominal wall with 0.1 cc. (10 R.U.) of follutein (Squibb). Theoretically a positive reaction was expected in all 4 rats, since prolan has not been demonstrated in the urine of any pregnant mammal below the primates. However, all animals gave negative reactions.

The negative results, combined with the difficulty of removing the hair and injecting the skin as well as the difficulty of reading the results because of the pinkness of the skin, prompted us to abandon the rat in favor of guinea-pigs and rabbits.

* From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland.

Here, however, we attained similar results and encountered the same difficulties. The monkey proved much more favorable from every standpoint.

Experiments on the Monkey. Much is known concerning reproductive phenomena in the rhesus monkey from the work of Corner, Allen, Hartman and others. The menstrual cycle is similar to that in women, but with a much larger porportion of non-ovulatory menstrual cycles. The period of gestation averages 164 days. After the first month of pregnancy Snyder and Wislocki found the urine free of prolan, as tested by the Friedman reaction. Recently, however, Hamlett demonstrated its presence in the urine from the 19th to the 25th day of the cycle. This rise probably corresponds to the rise of prolan in pregnant women, with this difference that in the monkey the amount drops suddenly below the level detectable by the Friedman test.

Sixteen monkeys, including 2 pregnant females, were used in the experiments. Injections were made into the skin of the anterior abdominal wall, which is white,

No.	Age, Years	Date Conception or Castration	Date of Test	Day of Cycle or of Pregnancy	Prolan used (R.U. in 0.1 c.c.)	Previous Injections
391	9	11–29–36	1–26–37	58 (preg.)	Antuitrin S (10)	
449	6	12– 6–36	1–27–37	52 (preg.)	Follutein (10)	
267	6½	—	1–27–37	8	Follutein (10)	
276	11	—	1–28–37	29	Follutein (10)	
384	8	—	1–26–37	18	Antuitrin S (10)	
396	6	—	1–28–37	37	Follutein (10)	
401	7	—	1–27–37	12	Follutein (10)	
534	5½	—	1–27–37	14	Follutein (10)	
256	9	—	2–16ff.–37	3	Follutein (10)	Follutein
338	8	—	2– 8–37 ff.	26	Follutein (10)	Follutein
17	13	2– 8–33	2– 5–37	—	Follutein (10)	Progynon and Gamone
330	7	12–14–36	1–27–37	—	Follutein (10)	Amniotin
258	6½	4–20–34	2– 5–37	—	Follutein (10)	
331	7	1–13–37	2– 5–37	—	Follutein (10)	
398	6	12–10–36	1–27–37	—	Follutein (10)	
76	8	12– 4–36	2– 5–37	—	Follutein (10)	

easily injected and readily observed. Antuitrin-S† was used twice and follutein (Squibb)† for the remainder of the tests. Whether 10 or 25 R.U. were used, 0.1 cc. of liquid was employed in each case.

The results of the tests on the monkeys are given in the accompanying chart. They were uniformly negative in non-pregnant or pregnant, normal or castrate. The only reaction seen was a pale weal 1.0 to 1.5 cm. in diameter around the point of injection. With 25 R. U. of follutein (Squibb) the weal measured up to 2.5 cm.

On the theory that a sensitivity might be produced in an animal by injection of hormones previous to the test the following experiments were performed: *1)* Monkey 330 received 35 R. U. estrone in the form of amniotin (Squibb) daily from Jan. 12 to Feb. 8. Since estrone causes an outpouring of gonadotrophic, chiefly luteinizing hormone, this procedure seemed somewhat equivalent to injection of prolan. A test on February 8 with 10 R.U. follutein proved negative. *2)* A similar experiment was made with monkey 17, which received 300 R.U. progynon-B (Schering) between Jan. 25 and 30. On Jan. 30 and 31 and Feb. 1 she received 25 R. U. gamone (Squibb)

† We make grateful acknowledgments to the following firms: To E. R. Squibb and Sons for the urinary prolan follutein, for the estrone amniotin and gamone, a gonadotropic hormone made from human castrate and menopausal urine; to the Schering Corporation for the dihydroestrin-benzoate, progynon-B; to Parke-Davis Co. for the urinary prolan antuitrin-S.

twice daily, for the following 3 days (Feb. 2, 3 and 4) 50 R.U. twice daily. On Feb. 5 a skin test was made with follutein and again there was no erthema whatever around the weal. *3)* Female 338 received 250 R.U. follutein intravenously on the morning of Feb. 8, day 26 of the menstrual cycle. A skin test made with 10 R. U. of follutein 5 hours later was again negative. The skin test proved negative also every day for a week. *4)* Female 256 was given 250 R.U. of follutein intravenously and 250 R. U. intraperitoneally on Feb. 15, 1936. Skin tests made each day for 6 days beginning Feb. 16 never showed the slightest erythema.

It is thus seen that all animal tests with follutein or antuitrin-S proved negative under varying conditions.

For purposes of control, particularly to test the potency of the hormone used on animals, skin tests were made on both pregnant and non-pregnant women through the courtesy of Dr. Cullen and Dr. Eastman of the Johns Hopkins Hospital. The tests were all made with follutein (Squibb) and the results may be summarized as follows.

Nineteen non-pregnant, normally menstruating women and 23 pregnant women were tested. In age the non-pregnant varied from 21 to 45 years and were in the hospital for treatment of inflammatory disease of the tubes or cervix or for removal of uterine myomata. Of the 19 non-pregnant women, 11 gave definite erythematous reactions varying from 2 to 4 cm. in diameter by the end of the first hour following injection. In 5 a lighter pinkish erythema appeared and in 1 patient the reaction was so slight that if she had stated that she had missed a period the diagnosis of pregnancy would have been made. Since the patient was definitely not pregnant, however, the case must be recorded as questionable positive reaction.

The ages of the 23 pregnant women ranged from 17 to 35 years. Their pregnancies varied between 16 and 40 weeks. No erythema appeared in 17, in 5 there was a slight erythema at the end of 1 hour and a half. In 1 patient, a 32-year old woman in the 18th week of pregnancy, a definite full red erythema was obtained, as Gilfillen and Gregg stated occurred only in the non-pregnant.

Summary. The intradermal skin test of Gilfillen and Gregg proved 100 per cent negative in pregnant and non-pregnant monkeys as well as in laboratory rodents. Injection of prolan or estrone did not sensitize the monkey to the reagent (follutein, Squibb). A small control series of 19 non-pregnant and 23 pregnant women demonstrated inferior reliability of the test as compared with the Aschheim-Zondek or Friedman test.

SOME OBSERVATIONS OF THE GONADOTROPHIC HORMONES OF PREGNANCY

BENJAMIN TENNEY, JR., AND FREDERIC PARKER, JR.*

In this work on the gonadotropic hormones of pregnancy we recognize the 3 distinct hormones that are now generally believed to be involved. Two of these hormones are considered to be of pituitary origin. The first, the follicle-stimulating hormone, or FSH, causes growth of ovarian follicles. The second, the luteinizing hormone or LH, causes the formation of corpora lutea from the ripened ovarian follicles. The third hormone involved is the prolan of pregnancy presumably of placental origin, commonly called the anterior pituitary-like hormone, or APL.

In pregnancy a positive Aschheim-Zondek test can be obtained as early as 10 days after conception or approximately as soon as the ovum becomes imbedded in the uterine wall. The maternal blood vessels are broken into by fetal cells. The secretion of these cells is absorbed into the maternal blood stream and excreted by the kidneys. When first recovered in the urine, such as in 1 case that was 4 days overdue and contained 40 R.U. of APL per liter, the effect on the ovary was primarily follicle-stimulating without definite corpus luteum formation. Six days later 166 R.U. was recovered from the urine and definite corpora lutea were formed. This is the explanation of certain doubtful Aschheim-Zondek tests found early in pregnancy and is most probably due to the small amount of the hormone present. The method of identification of APL is that in hypophysectomized rats it will not form corpora lutea but will cause enlargement of the ovary by luteinization and growth of ovarian stroma. This is in contrast to pituitary extract which causes growth of corpora lutea in hypophysectomized rats. Injections of early pregnancy whole urine in hypophysectomized rats cause typical stroma growth and luteinization without any corpora lutea. In addition, if this APL is extracted from whole urine by precipitation with 5 times the volume of alcohol and the amount assayed on immature rats, it will be found that the entire amount of this hormone has been precipitated.

In contrast to early pregnancy, studies made on patients at term or during labor show certain marked differences. Specimens of whole urine will give a positive Aschheim-Zondek reaction in immature rats in no way different from that of early pregnancy. On treatment of the urine with alcohol less than 20 per cent of the hormone will be precipitated. Injection into hypophysectomized rats of whole urine at term gives no reaction whatsoever and neither does the alcohol precipitate. This lack of reaction in hypophysectomized rats of full-term urine is not due to dilution or absence of hormones because the hypophysectomized rats were injected with 10 to 15 R.U. according to the titre of the whole urine. In contrast to the urine, extracts of full term placentas gave a typical APL reaction in hypophysectomized rats. We have found that this APL of the full term placenta will precipitate out from 50 to 100 per cent. Therefore, it appears that although there is APL in the placenta at term it is being excreted in very small amounts.

This brings up the question of just what is the substance that is present in normal

* From the Mallory Institute of Pathology, Boston City Hospital, Boston, Massachusetts.

full term urine that is not precipitated by alcohol and that acts on immature but not on hypophysectomized rats. It is not primarily APL because this is precipitated by alcohol and acts on hypophysectomized rats. Our only answer is to call this a pituitary-stimulating substance that appears in considerable amounts at term in normal cases. It gives neither the reactions of pituitary nor placental gonadotropic hormones. If this is a definite substance that does not precipitate out with alcohol, then it must be present in the supernatant fluid left after precipitation. To test this, the alcohol was removed from the supernatant fluid by evaporating under lowered pressure. Injection of this alcohol-free supernatant fluid in immature rats gave a definite FSH reaction. Injection into hypophysectomized rats gave no reaction. Therefore this is definitely a pituitary-stimulating substance. There is the possibility that the above reactions are due to the presence of estrogenic substances in the supernatant fluid. We do not feel this to be the case as this pituitary-stimulating substance

	Immature rats per cent APL in alcoholic precipitate	Reaction on Hypophysectomized rat
Early pregnancy urine	100	Marked P.U.
Full term normal urine	10– 20	None
Full term normal placenta	50–100	Marked P.U.
Full term toxemic urine	100	Marked P.U.

can be destroyed by boiling. The alcohol-free supernatant fluid was boiled for 10 minutes and injected into immature rats. These rats gave no reaction to this injection. It is well known that boiling estrogenic substances in the urine increases the amount that will give a physiologic reaction.

The study of the urine of toxemias has proved of considerable interest. We have injected whole urine and the alcoholic precipitate into both immature and hypophysectomized rats. This work was somewhat more difficult as there is some substance in toxemic urine that is extremely toxic to rats, especially the hypophysectomized rats whose resistance is poor. The findings were of interest in that the gonadotrophic hormones present showed a definite APL reaction in hypophysectomized rats. This is in contrast to the normal cases at term which give no such reaction. This was true in both the whole urine and the alcoholic precipitate. Also in these toxemic urines practically 100 per cent of the hormone was present in the alcoholic precipitate. Therefore in the toxemias there seems to be a prolongation of the excretion of APL over the normal. As far as can be told the reactions of this APL are identical with that of early pregnancy but it is its presence and amount in the urine that is the abnormal finding. From the 100 per cent precipitation of the toxemic urine it would appear that there is an absence of the pituitary-stimulating substance found in normal full term cases.

Summary. In early pregnancy the gonadotrophic hormone recovered from the urine is almost entirely of placental origin as shown by its reaction on hypophysectomized rats and its complete precipitation by alcohol. In normal cases at term this APL has practically disappeared from the urine and a pituitary-stimulating substance is present which will act on immature but not hypophysectomized rats. In toxemias at or near term there is an increased amount of APL which can be recovered from the urine with a decrease or absence of the pituitary-stimulating substance present in normal cases.

THE BITTERLING FISH RESPONSE TO MALE SEX HORMONES*

SEWARD E. OWEN

The male bitterling fish (Rhodeus amarus) develops the typical mating coloration when treated with female sex hormones. Logically it would appear that the female fish might display the spawning season changes, when treated with male sex hormones.

The Japanese bitterling (Achelognathus intermedium) was employed in the study here reported.

Methods. The males were stocked separately from the females. In the test, individual aquaria containing 1 liter of water were used. The water temperature was 20 C. and the pH 6.53. Materials tested were added directly to the water or painted on the food particules.

In interpreting results a lengthening of the external ovipositor in the female to at least 3 mm. was regarded as positive. In the male fish two of the following changes were considered as positive response: development of a red tip or red edge on the dorsal fin; production of a red cast to the eyes; the appearance of a red orange tip or reddish edge on the anal fin; the development of a pinkish body cast or flush.

It was noted that the externally visible reactions regressed when the fish were again placed in fresh water. Such fish may safely be used again later. No fish were kept in the test aquaria longer than 7 days.

Results. With testosterone acetate 100 per cent of the male fish developed positive nuptial coloration on total dosages as low as 0.04 I.U. Dosages as high as 35 I.U. were essential to affect 66 per cent of the female fish positively, while dosages as low as 1 I.U. never influenced the female fish.

Aqueous testicular extracts produced positive reactions in 50 per cent of both males and females provided the fresh testicular equivalent of the daily dose was 0.702 gm. The daily feeding of 5 mg. of desiccated testes resulted in positive reactions in the male fish but produced no effect upon the females. Oil extracts of testis with a total dosage equivalent of 10 gm. of testis resulted in positive coloration in the males but had no effect on the female fish.

Desiccated anterior pituitary fed in 5 mg. lots daily produced 66 per cent positives in males and some effect in 60 per cent of the females although the ovipositors did not reach 3 mm. in length. Some growth factor extracts of anterior pituitary were negative on males but occasionally induced positive reactions in females. The effective and toxic doses were quantitatively similar. The female fish did not respond to antuitrin-S, and lactogenic extracts were not employed.

Suprarenal substance as a powdered food produced an atypical blue-green body coloration in both males and females. The female fish occasionally displayed some

* From the Cancer Research Laboratories, U. S. Veterans' Facility, Hines, Illinois.
Published with the permission of the Medical Director of the Veterans' Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

ovipositor lengthening. Changes due to this substance, however, were not interpreted as true positives.

The urine from an aged male patient receiving routine male endocrine injections for prostatic hypertrophy, induced an occasional ovipositor lengthening in female fish but no effect on male fish was noted. The urine from a totalily castrate man was ineffective on female fish.

The female controls fed rice food or to whose aquaria the oily solvents were added were routinely negative. It was noted that one brand of powdered shrimp fish food caused the male fish to show positive coloration but no positives in female fish were induced by this food.

Summary. It is difficult to break ovipositor dormancy in female bitterling fish by treatment with the sex hormones. The nuptial coloration in male bitterling fish may be produced rather easily by means of several endocrine preparations.

In the search for biological test objects for endocrine preparations, it appears that more knowledge of the chromatophore reactions and ovipositor changes in fish is essential before these may be used for specific endocrine assay purposes. The normal factors associated with the spawning season changes must be studied; among these are hydrogen-ion concentrations, temperature levels, radiant energy factors and others.

THE OCCURRENCE OF ESTROGENIC SUBSTANCES IN THE OVARIES OF ECHINODERMS

J. KENNETH DONAHUE AND EARL DEWITT JENNINGS*

Although it has become increasingly evident that estrogenic substances are widely distributed in nature,[1] the search for such substances in the gonads of marine forms has only recently been inaugurated. Extracts of the ovaries of the swordfish have been shown to contain demonstrable quantities of what appears to be the female sex hormone.[2] With the object of determining whether or not the gonads of marine forms of a lower order also contained estrogens, the present investigation was carried out.

Experimental. The ovaries of the species Lytechinus variegatus were used for testing purposes. The echinoderms were collected from the waters of Bermuda during the breeding season (August) at which time the ovaries were swollen with ripe follicles. The ovaries were dissected out, thoroughly ground in a mortar, and placed in separate containers of 90 per cent ethyl alcohol. Extraction was done in Charleston late in September. The extraction procedure was essentially that described by Allen and Doisy[3] involving repeated extractions with 95 per cent alcohol, concentration by distillation, followed by repeated extractions with ether and subsequent concentration. The residue distributed itself into a light, oily layer called here the light fraction, and a heavy, gummy layer called here the heavy fraction. Since no attempt was made to obtain quantitative data in these initial experiments, the partially purified product obtained by this method was considered adequate for the

* From the Departments of Biology and Chemistry, The College of Charleston, Charleston, South Carolina, and The Bermuda Biological Station for Research, Inc., St. Georges, West, Bermuda.

[1] Allen, E.: Sex and the Internal Secretions. Chapter X, Williams and Wilkins, Baltimore, 1932.
[2] Weisman, A. I., D. I. Mishkind, I. S. Kleiner, and C. W. Coates: Endocrinology 21: 3. 1937.
[3] Allen, E., and E. A. Doisy: J.A.M.A. 81: 819. 1923.

purpose. The extracts were taken up in corn oil for purposes of injection. Mature, female rats were used for testing purposes.

Results. Six mature female rats were ovariectomized and left untreated for 10 days. During that period daily diestrous smears were obtained. Then 3 were injected subcutaneously with 0.3 cc. of light fraction extract daily for 10 days. The remaining 3 animals received injections of heavy fraction at the same dosage level. In the light-fraction group the smears taken daily during the injection period showed much mucous, a markedly sparse number of leucocytes, and scattered small clumps of nucleated and cornified epithelial cells. The smears of the heavy-fraction group were typically diestrous in character throughout the injection period. The animals were killed 24 hours after the last injection and sections made of the vaginae and uteri. In 2 of the 3 animals receiving the light fraction, active growth, as evidenced by numerous mitotic figures, was noted in the germinal layers of both uteri and vaginae. The cells newly added to the walls of the vaginae were clearly distinguishable as a darkly stained band of tissue 6 cell-layers in thickness and contrasting markedly to the lightly stained old vaginal walls. The third animal showed less evidence of active growth in the vagina and uterus, a few scattered mitotic figures being seen in the sections of the vagina examined. The uteri and vaginae of the heavy fraction group presented the characteristic histological picture of castration atrophy. Each animal received the equivalent of 4.2 gm. of dried ovarian tissue during the 10-day injection period.

Discussion. The positive reactions of 2 of the animals receiving light fraction can be considered only a threshold response since full estrous smears were not obtained. However, the method of extraction used in this instance very probably yielded less than 50 per cent of the total amount of estrogenic substance actually present in the original material. It is estimated that at least 1 R.U. is present in 4.2 gm. of dried echinoderm ovary. It is clearly indicated that histologic examination of the reproductive tract is a necessary adjunct to the smear technique when testing for minute amounts of estrogens.

Summary. An extract of the ovaries of a Bermuda echinoderm (Lytechinus variegatus) caused considerable growth of the vaginal and uterine endometria in 2 of 3 spayed female rats. It is estimated that 4.2 gm. of ovarian substance (dry weight) contain at least 1 R.U. of estrogenic substance.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
I. Andrussier
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

Clinical, hormonal and histological conditions in a case of hermaphroditism. Charvát, J., E. Kodíček and O. Schubert. To appear in this Journal.

The history of a case of hermaphroditism (female) is reported. The amount of gonadotropic hormone in the urine, titrated during 1 month, was small. The estrogenic hormone was secreted in an increased quantity, however, as was also the male hormone. The ovary was small with numerous immature follicles, without the slightest trace of luteinisation. The uterus was small and hypoplastic, with an atrophic mucous membrane. The adrenals were slightly enlarged with cortical adenomas. Basophilic cells in the hypophysis were proliferating. In spite of the increased proportion of estrogenic hormone the uterus was in a state of complete inactivity. The authors also report considerable amounts of male hormone in other cases of hirsutism in women, even amounting in some cases to more than that secreted by normal males.

Observations on the quantitative assay of growth-promoting extract of the hypophysis. Chou, C., C. Chang, G. Chen and H. B. Van Dyke. To appear in this Journal.

Observations on quantitative aspects of the assay of the growth-promoting hormone of the pituitary were made in groups of rats, the totals of which were 288 normal adult females, 130 completely hypophysectomized rats (duration of hypophyseal deficiency 1 to 10 months) together with 29 partially hypophysectomized rats and 58 littermate control animals. All the assays were performed with a single extract of the pars glandularis of the ox pituitary. In terms of the percentage increase in weight, the sensitivity of the hypophysectomized rat is probably more than twice that of the normal adult female. Likewise variation in dose produces a clearer difference in response in the hypophysectomized rat. In the normal rat, unlike the hypophysectomized, a dose-variation of 100% often does not produce a convincing difference in response. There is no sexual difference in the sensitivity of the hypophysectomized rat. The normal female is more sensitive than the male. The optimum period over which daily injections should be given is about 10 days. It appears to be the same in normal or hypophysectomized animals. The response of both normal and experimental animals after a rest period of 2 to 6 weeks was less. The reduction in response appeared to be greater the larger the dose of the first course. The loss of weight following injections is variable but similar in both normal and hypophysectomized rats.

The excretion of androgenic and estrogenic substances in the urine of children. Dorfman, R. I., and W. W. Greulich. To appear in this Journal.

The urines of 18 boys and 5 girls, ranging in age from 6 to 16 years, were assayed for androgenic and estrogenic activity. The androgenic activity varied from 1.1 to 32 International Units per 24 hours, while the estrogenic activity ranged from less than 5 to 95 I.U. for the same period. Attention is called to the difference in the sex hormone excretion of children who, though of the same chronological age, differ from each other in the degree of their physical maturity.

The effect of splenectomy on the weight of the hypophysis of the albino rat. Edwards, L. F., and C. W. Wright. To appear in this Journal.

One hundred female albino rats of various ages were used in this experiment; 50 were splenectomized and 50 served for controls, the latter being litter mates of the former. The post-operative period arbitrarily chosen between operation and cancellation was two weeks in all cases. The mean hypophysis weight of the operated animals was 25% greater than that of the controls. The mean value of the relative percentage of hypophysis weight to body weight of the operated group was 27% greater than that of the control group. Statistical treatment of the data obtained in this study brings out the following facts: (1) that the difference in weight between the hypophysis of the splenectomized animals and that of the controls is significant and is not due to random sampling; (2) that the difference in body weight of the two groups of animals is not significant.

Responses of the human ovary to gonadotropic principles. Hamblen, E. C., and R. A. Ross. To appear in this Journal.

An analysis of observations upon 85 patients after gonadotropic therapy and detailed reports of the ovarian responses of 7 patients is made. Six of the 7 cases reported in detail were of patients in whom presumptive diagnoses of anovulatory failure had been made. The total doses in these patients ranged from 6000 to 24,250 R.U., the daily doses varying from 400 to 8000 R.U. The observed ovarian responses, judged either by direct inspection of ovaries, by microscopic study of these, or by inferential studies of the endometrium, do not warrant an assumption that ovulation and corpus luteum formation had resulted from such therapy.

The gonadotropic and the gonadotropic-like factor in the treatment of male sterility. Heckel, N. J. To appear in this Journal.

In selected patients in whom there is a disturbance of spermatogenesis, both the gonadotropic principle and extract of pregnancy urine appear to influence the number and viability of spermatozoa. In some patients, however, the phenomena are only temporary. Clinical observations on 20 sterile male patients are reported. Of the two hormones the gonadotropic principle was no more efficacious than was the extract of pregnancy urine. The limits of fertility, as revealed by spermatozoa counts where pregnancies did occur are somewhat lower than have been reported by others. There appeared to be an optimum period of spermatogenesis in patients who had been under treatment for as long as a year with the gonadotropic principle. After this period, continued treatment caused a recession in the number of spermatozoa. An additional observation was that whereas the anterior pituitary-like principle has been observed to cause a growth of the external genitalia in boys of prepuberal age, no similar growth occurred in these male adults.

Testosterone and testosterone acetate and the protein and energy metabolism of castrate dogs. Kochakian, C. D. To appear in this Journal.

The subcutaneous injection into 2 castrate dogs of oil solutions of crystalline testosterone and testosterone acetate resulted in a decreased protein metabolism which was demonstrated by a decreased excretion of urinary nitrogen. The total energy metabolism was not affected. The results are similar to those obtained in previous studies with urinary male hormone extracts and androstenedione.

The effect of graded doses of estrin upon the pituitary, adrenal, and thymus weights of mature ovariectomized rats. Lauson, H. D., C. G. Heller and E. L. Sevringhaus. To appear in this Journal.

The average pituitary and adrenal weights of 67 mature female albino rats were not changed by a 20-day period of castration, when compared to those of 36 normal females of the same age and weight range. Twenty days of castration led to marked hypertrophy of the thymus gland. Injection of 0.25, 0.50, and 5.0 gamma of dihydroxyestrin (3.1, 6.2, and 62.5 R.U.) daily for 19 days immediately following castration caused distinct pituitary hypertrophy averaging 28, 32 and 46% above the level of normal and castrate controls. Treatment with these doses of estrin did not alter the adrenal weights. The two smaller doses of estrin prevented castration hypertrophy of the thymus, while the large dose caused atrophy of the gland 25% below normal level. Estrin injection of castrates prevented some of the body weight increase characteristic of the spayed rat.

Alterations in the percentage of cell types in the hypophysis by gonad transplantation in the rat. Pfeiffer, C. A. To appear in this Journal.

The physiology of the hypophysis of the rat was altered by castration at various ages and by grafting gonads into both sexes either castrated or normal. This physiology was then checked with the percentage of eosinophiles and basophiles in the pituitary, and it was found that for a particular physiological alteration there was a corresponding change in the cell percentages. Males castrated at birth and receiving ovaries at birth or at puberty had the female type of pituitary both in physiological response and in cell counts, while females castrated at birth and receiving testes at that time had pituitaries which correspond to that of the male in physiology and cell pattern. If both testes and ovaries were present during the prepubertal period, the testes dominated and influenced the differentiation toward the masculine type. After puberty there is a distinct and separate pituitary pattern for each sex, which appears to be so undifferentiated in the early prepubertal stage that one pattern may be changed into the other by the presence of the gonads of the opposite sex. However, attempts to change one pattern to the other after complete differentiation into the two distinct types (after puberty) have been unsuccessful even though both the physiology and histology may be altered to a certain extent within the limits of the pattern.

The urinary excretion of sodium pregnanediol glucuronidate in the human menstrual cycle. Venning, Eleanor H., and J. S. L. Browne. To appear in this Journal.

The quantitative excretion of sodium pregnanediol glucuronidate in the human menstrual cycle in 10 cases is reported. This compound is considered to be an excretion product of progesterone. It is excreted only in the luteal phase of the menstrual cycle and appears in the urine within 24 to 48 hours after ovulation. The excretion of this compound is continuous for a certain period, varying from 3 to 12 days in the cases studied; in 6 of the 10 cases the period was 10 to 12 days. Bleeding occurs from 1 to 3 days following its disappearance from the urine. The total amount of pregnanediol excreted per period varied from 3 to 54 mg. The measurement of this compound in the menstrual cycle offers a means of determining the duration and functional activity of the corpus luteum.

ADRENALS

Maintenance of adrenalectomized dogs without cortin, through control of the mineral constituents of the diet. Allers, W. D., and E. C. Kendall, Am. J. Physiol. **118:** 87. 1937.

Two adrenalectomized dogs were maintained without cortin for 84 and 115 days by the addition of sodium chloride and sodium citrate to the diet. Three other adrenalectomized dogs were maintained by sodium chloride and sodium citrate alone except during crises when cortin was required. The diet consisted of casein, sucrose, lard, butter fat, agar agar or bone ash and yeast. Occasionally canned dog food or lean meat were given to please the appetite. The concentrations of urea, glucose, sodium, potassium, chloride and bicarbonate in the blood were all within normal limits.—F. A. H.

Studies on the opossum (Trichosurus vulpecula). The effects of splenectomy, adrenalectomy and injections of the cortical hormone. Anderson, Doris, Australian J. Exper. Biol. & M. Sci. **15:** 24. 1937.

Following splenectomy the blood sugar and total blood phosphorus were unaltered up to 2 months following the operation. The non-protein nitrogen was nearly doubled, the total lipoids, the fatty acids and the cholesterol were lowered by approximately 40% and the lipoid phosphorus by 20%. The kidney and adrenal lipoids were unchanged but in the liver the cholesterol and lipoid phosphorus were lowered by about 25%. Unilateral adrenalectomy led to an increase in weight of the remaining adrenal, together with a higher total lipoid and cholesterol content in the gland. The liver showed an increased cholesterol content. All the animals subjected to bilateral adrenalectomy died within 26 to 40 hours after the operation; the animals was therefore regarded as being hypersensitive to total adrenalectomy. The lipoid constituents of spleen and kidneys were found to be unchanged but the liver showed a raised cholesterol content. Injections of 1 cc. of eschatin hourly for 3 hours (the equivalent of 120 gm. of fresh adrenal cortex) caused a marked rise at the end of the fourth hour in the blood sugar. The ratio between cells and plasma of the non-protein nitrogen was lowered, and the lipoid phosphorus and fatty acid content of the liver and spleen were raised.—G. B.

Addison's disease due to suprarenal atrophy. Anderson I. A., and A. Lyall, Lancet **232:** 1039. 1937.

Cases of Addison's disease associated with thyrotoxicosis have been rarely reported, although the thyroid gland does show evidence of pathologic changes. The authors report a case of Addison's disease due to suprarenal atrophy and preceded by thyrotoxicosis in a woman 30 years of age, who died in hypoglycemia. The patient presented other interesting features: she had a strong distaste for salty food; the crisis appeared immediately following a menstrual period. This relationship between menstruation and increased severity of symptoms has been commented on by several authors. The increase in suprarenal insufficiency is presumably due to the added stress of the menstrual period which increases the need of the body for the cortical hormone. The plasma chlorides and blood urea were both within normal limits but there was extreme hypoglycemia. The authors maintain that the coma and muscular spasm is directly related to hypoglycemia.—L. B. S.

Intra-arterial acetylcholin in sympathectomized subjects (Acción de la acetilcolina intra-arterial en sujetos simpatecto-mizados). Battro A., and A. Lanari, Rev. Soc. argent. de biol. **12:** 454. 1936.

Eleven subjects, in whom a lumbar or cervical sympathectomy had been performed a few days to 4 years previously, received 0.04 gm. acetylcholin in the artery of the denervated limb. In all cases pain and flushing occurred; in some, vasoconstriction was seen and no sweating appeared; in others there was vasodilatation and sweating as in normal subjects.—J. T. L.

Adrenin content of the adrenal glands in dogs subjected to decreased barometric pressure (Taux d'ardrénaline dans les capsules surrénale, chez les chiens soumis a la dépression barométrique). Binet, L., and J. Lanxade, Compt. rend. Soc. de biol. **122:** 1011. 1936.

Five dogs were placed under conditions simulating altitudes of from 26,000 to 40,000 feet for 43 minutes. Their adrenals contained on the average 2.55 mg. of adrenin per gm. of fresh tissue. The normal average is 1.44 gm.—J. C. D.

Further observations on sodium chloride balance in the adrenalectomized opossum. Britton, S. W., and H. Silvette, Am. J. Physiol. **118:** 21. 1937.

The influence of fasting, adrenalectomy and dehydration by diarrhea on sodium levels was studied in normal and lactating opossums. There appeared to be a delicate salt balance in this animal so that adrenal insufficiency and dehydration were followed by a rise of serum sodium and chloride in contrast to the decrease observed in mammals. Withholding food and water from adrenalectomized opossums was followed by normal or slight increase in sodium and chloride levels while withholding of food alone resulted in markedly diminished serum levels and less marked depletion in serum chloride concentration. Extrarenal loss of salt (by lactation and diarrhea) produced similar chloride and greater sodium reduction. Adrenal insufficiency was accompanied by lowered serum sugar and liver and muscle glycogen. The authors conclude that serum sodium chloride levels are reflections of the rate of renal or extrarenal excretion and not to redistribution of the salt.—R. R. Durant.

A comparison of sodium, chloride and carbohydrate changes in adrenal insufficiency and other experimental conditions. Britton, S. W., and H. Silvette, Am. J. Physiol. **118:** 594. 1937.

Following adrenalectomy in the cat the serum sodium and chloride were observed to fall in the ratio 1.26, while the average following other abdominal operations was in the ratio 0.59. The serum sugar rose following operations except in the case of adrenalectomy.—Lena Lewis.

Studies on the physiology and pathology of the adrenals; cortical hormone and exercise in Addison's disease (Estudios de fisiopatologia suprarrenal; hormona cortical y ejercicio en la enfermedad de Addison). Collaz, J. A., and L. Hernández, An. de med. int. **4:** 849. 1935.

In 5 cases of Addison's disease the authors studied the influence of the cortical hormone upon the blood lactate curve after exercise. In such patients the lactate curve is slow in receding, but after cortical hormone treatment it becomes normal.—G. Marañon.

Observations on the chemical determination of adrenalin. J. Devine, Biochem. J. **31:** 545. 1937.

The Folin phenol-reagent, for the determination of adrenalin, reacts with uric acid

and especially with ascorbic (cevitamic) acid, both of which may be present in glandular extracts; the depth of color obtained also depends on the pH at which it is developed, and these errors may cancel out. Adrenal extracts contain an unidentified catechol derivative, which has no pressor effect, does not affect the Folin reagent, and like uric and ascorbic acids does not interfere with the reactions in which adrenalin is oxidized to a red compound.—D. L. T.

Bronze diabetes and adrenal insufficiency (Diabète bronzé et insuffisance surrénale). Labbé, M., R. Boulin and Ullmann. Presse méd. **44:** 1769. 1936.

Diabetic patients showing skin pigmentation suggestive of Addison's disease have variably a high or a low resistance to insulin. They are inclined to asthma and have a low blood pressure. The sodium chloride in the serum of a case described was sub-normal by 10%, the glutathione by 50% and the potassium increased by 5%. The varied pathological findings in bronze diabetes are discussed.—A. E. Meyer.

The histology of the mammary glands of adrenalectomized lactating rats. Levenstein, I., Anat. Rec. **67:** 477. 1937.

The litters of adrenalectomized mothers are not able to survive to the weaning time. This is due to a deficiency in the secretory activity of the mammary glands of the mothers. The histological picture of these glands shows only slight structural changes from normal secreting glands. Secretory elements are present and functional. The most striking difference is that the alveoli are less distended and apparently contain a more dense secretion. Although the litters of untreated adrenalectomized rats generally die of starvation, milk secretion does not completely stop as long as the litters are still suckling. The normal histological picture of the mammary glands as well as their function can be, in most cases, largely restored by salt therapy. An analogous condition to that found in the mammary glands of operated animals can be brought about by depriving normal lactating animals of fluids. These results are interpreted as a substantiation of the theory that the deficiencies following adrenalectomy are due largely to the disturbance in water and salt balance.—Author's Abst., Wistar Bib. Service.

Homeotransplantation of the adrenal gland of the newborn rabbit following growth of the gland in vitro. Lux, Lydia G., M. Higgins and F. C. Mann, Anat. Rec. **67:** 353. 1937.

Adrenal glands removed from newborn rabbits were grown in vitro in Carrel flasks. Healthy adult male rabbits were selected as hosts and plasma and serum of these animals were used as the media of the cultures. The cultures were incubated at 37.5° for 2 weeks to 18 days. The fragments with growth and clot were aseptically removed from the flasks and transplanted into the groin of the host from which the serum and plasma had been taken. About 80 to 100 fragments were transplanted in this way to each animal. These animals were not adrenalectomized. Six animals comprise this series. Exploration of the site of transplantation was made at 2, 4, 6, 8 and 12 weeks after operation and portions of the tissue were removed for fixation and study. The reaction by the host to the adrenal fragments proved to be similar to the reaction to any foreign body. Histological observations indicated a marked cellular infiltration and a walling-off of the fragments by dense fibrous bands of connective tissue. Degeneration of the fragment and lymphocytic infiltration had started at 2 weeks. After 12 weeks the fragments of gland were completely destroyed and there was a marked invasion of lymphocytes, histiocytes and polymorphonuclear leukocytes. The fragments were usually well vascularized.—Author's Abst., Wistar Bib. Service.

Influence of adrenalectomy on the ketosis of fasting and on the action of the anterior pituitary ketogenic principle. MacKay, E. M., and R. H. Barnes, Am. J. Physiol. **118:** 184. 1937.

The authors have presented further evidence of the reduction or abolition of ketosis of fasting on anterior pituitary origin, after adrenalectomy in the rat. In all, 26 female animals were used. Ketone bodies in urine and blood were determined by the method of Van Slyke. Anterior pituitary principle was obtained from the Wilson Laboratories (Phyone) or prepared according to the method of Best and Campbell. Fasting ketosis in the female rat when at a low level following a stock diet or at a high level following fat feeding is reduced by bilateral adrenalectomy. Ketosis due to ketogenic anterior pituitary extracts as a rule is reduced or does not occur after removal of the adrenal. When this preparation shows ketogenic properties in adrenalectomized rats they nearly always survive for a long period without therapy, evidence that removal of adrenal cortex was not complete.—K. Brownell.

Fasting ketosis in the pregnant rat as influenced by adrenalectomy. MacKay, E. M., and R. H. Barnes, Proc. Soc. Exper. Biol. & Med. **34**: 682. 1937.

Starvation produced a much greater ketosis in pregnant than in nonpregnant rats. The removal of the adrenals largely prevented this starvation ketosis of pregnancy.—M. H. F.

Sexual function in chronic adrenal insufficiency (La fonction sexuelle dans l'insuffisance surrénale chronique). Marañon, G., Presse méd. **44**: 2058. 1936.

Among women developing Addison's disease in later life 38.6% reported a delayed menarche, 54.5% had oligomenorrhea and 4.5% became amenorrheic before 30 years. The fecundity was low but the incidence of abortion was not unusually high. Toxic conditions of pregnancy prevailed. The lactation was usually deficient. The hypoadrenal man is hyposexual; 11.1% were impotent, 24.7% frigid. The mortality of the offspring was abnormally high. Men with latent Addison's disease showed frequently a feminine type.—A. E. Meyer.

Basal metabolism in chronic adrenal deficiency; action of adrenal hormones (cortical and medullary) upon metabolism (Le métabolisme basal dans l'insufissance surrénale chronique; action des hormones surrénales [cortical et médullaire] sur le métabolisme). Marañon, G., L. Calvo Pena and E. Ossorio Florit. Ann. de méd. **37**: 168. 1935.

The authors have studied the basal metabolic rate in 51 cases of Addison's disease. In 23.5%, only, was hypometabolism observed. In 66.6% the metabolism was normal and in 9.1 it was above normal. The basal metabolic rate, therefore, is of little diagnostic significance. In these cases injections of pure cortical hormone caused a decrease in the basal metabolic rate, confirming observations of other workers. Injections of adrenalin increased the rate.—Authors' Abst.

Creatine metabolism in Addison's disease (Estudios de fisiopatologia suprarrenal; metabolismo de la creatina e la e fermedad de Addison. Su exploración por las pruebas de la ingestión de glicocol y creatina). Marañon, G., J. A. Collazo and A. Fernández Cruz, An. de med. int. **4**: 377. 1935.

The administration of glycocol by mouth to Addisonians produced a temporary increase in the elimination of creatine, and a lasting increase in the output of creatinine and phosphorus. The administration of creatine augmented the elimination of creatinine.—Authors' Abst.

Mineral and water metabolism in Addison's disease. The influence of sodium chloride treatment in muscle metabolism in Addison's disease (Metabolismo mineral y del agua en la enfermedad de Addison; el quismo muscular bajo la influencia del tratamiento del cloruro de sodio en los addisonianos). Marañon, G., J. A. Collazo and C. Pérez Moreiras. Arch. de med., cir. y especialid. **38**: 519. 1935.

By means of biopsy of deltoid muscle a study was made of the muscular metabolism in normal individuals and in Addisonians untreated and in these same Addisonians after treatment with NaCl. In the treated patients the muscle glycogen was increased in 18% of the cases in comparison with the level in normal individuals and in 96% of the cases compared with Addisonians before treatment. There was less variation in the lactic acid and the muscular phosphagen. It seems, therefore, that the cortical hormone normalizes the water and electrolyte content of the tissues and plasma.—Authors' Abst.

Adrenalin and blood potassium (Adrenalina y potasio sanguíneo). Marenzi, A. D., and Rebeca Gerschman, Rev. Soc. argent. de biol. **12**: 424. 1936.

Intravenous injection of adrenalin produced an increase in blood plasma K lasting a few minutes and followed by a decrease below the initial level. The increase was proportional to the dose of adrenalin up to a maximum of 3 to 8 mg. % produced by 0.05 mg. adrenalin. Blood cells K did not vary, neither did Ca, Na or Cl of plasma. By means of experiments in which the incoming and out flowing blood was analyzed it was shown that the liver discharged K which was rapidly fixed by the muscles. Hepatectomy prevented this action of adrenalin, but removal of the pancreas, spleen, adrenals, adrenal medulla, digestive tract, thyroid, carotid sinus and section of the vagi did not modify it. Cocain increased it, ergotamin and yohimbin decreased it and it was not modified by atropin or eserin.—J. T. L.

Corticoadrenal insufficiency: metabolism studies on potassium, sodium and chloride. Nilson, H. W., Am. J. Physiol. **118**: 620. 1937.

Electrolyte balance studies and blood electrolyte studies were carried out on 3 adrenalec-

tomized dogs and 1 control dog. Similar symptoms of adrenal insufficiency were produced by either a high potassium diet or a diet low in sodium. The concentration of potassium in the cells was found to increase immediately preceding crisis and to return to normal levels on recovery. The serum sodium and chloride and blood urea and hematocrit showed varying changes, not characteristic.—Lena Lewis.

Antithyroid effect of adrenal cortex (Zur antithyreoiden Wirkung der Nebennierenrinde). Oehme, C., Klin. Wchnschr. **15:** 512. 1936.

In guinea pigs receiving 0.1 mg. of thyroxin daily, the simultaneous injection of 1cc. of adrenal cortical extract (Cortidyn; a commercial preparation) markedly inhibited the usual rise of metabolism, and permitted the animals to live with little or no weight loss for 38 days, instead of succumbing with emaciation in 2 to 3 weeks. The extract used was vitamin-A-free and tyrosin-free, and the ascorbic acid content was too low to be considered. The active principle of the extract was destroyed by oxygen at 38°. In high doses, ascorbic acid (20 mg. daily) also protected against thyroxin, as did 30 mg. of di-iodo-tyrosin. The adrenal extract also prevented the metabolic response to thyrotropic extract, although it did not alter the histological picture of the simulated thyroid. The author discusses the paradox of the decrease in B.M.R. following adrenal ablation, but cites the literature in support of the thesis that there is an antagonism between the adrenal cortex and the thyroid; viz., the cortical hypertrophy under thyroid feeding, prolonged survival of adrenalectomized rats in which thyroidectomy had been performed, and the decrease in B.M.R. in thyrotoxicosis following administration of cortical extract.—M. H. F.

Further studies on the action of individual members of the vitamin-B complex on the increase in volume of the adrenal cortex induced by muscular activity (Weitere Untersuchungen über die Wirkung einzelner Glieder des Vitamin-B-Komplexes auf die durch körperliche Arbeit bedingte Volumzunahme der Nebennierenrinde). Perjés, J., Arch. f. d. ges. Physiol. **238:** 341. 1936.

Neither flavin nor the anti-neuritic vitamin B, when administered to rats, prevented the adrenal cortical hypertrophy which results from muscular activity. Autoclaved brewer's yeast, on the other hand, prevented this hypertrophy.—A. G.

Effect of small doses of salt on the survival period of young rats following bilateral adrenalectomy (De l'influence de petites quantités de sel sur la durée de vie de jeunes rats décapsulés des deux côtés). Schultzer, P., Compt. rend. Soc. de biol. **122:** 976. 1936.

In 50 gm. adrenalectomized rats, the average survival period was 5.7 days (77 animals). When 1 cc. of a 0.9% sodium chloride solution was injected, this period rose to 7.7 days (30 animals). If this dose was doubled, the period was increased to 9.3 days (11 animals). It is pointed out that in standardization of cortical extracts by the survival method, the possible effects of contamination with salt must be considered.—J. C. D.

Comparative influence of epinephrin and of dextrose on the utilization of sugar by the muscles, determined with the aid of thermostromuhr measurements of blood flow. Soskin, S., H. E. Essex, J. F. Herrick and F. C. Mann, Am. J. Physiol. **118:** 328. 1937.

The investigation was undertaken in order to determine, under better controlled conditions, the influence of epinephrin on sugar utilization by the muscle. Eleven dogs, anesthetized with sodium amytal, were used in the study. A Rein thermostromuhr was placed on the femoral vein of one hind leg. Continuous injections of dextrose or epinephrin were administered through the jugular vein by means of a Woodyatt pump. Sugar was determined on arterial and venous blood samples by the method of Folin. The work yielded no evidence that epinephrin decreases the utilization of sugar by the muscle.—K. Brownell.

Intake of potassium, an important consideration in Addison's disease. A metabolic study. Wilder, R. M., E. C. Kendall, A. M. Snell, E. J. Kepler, E. H. Rynearson and Mildred Adams, Arch. Int. Med. **59:** 367. 1937.

The potassium content of the diet of patients with Addison's disease affects the course of the disease and the development of the symptoms of crisis. An intake of K not greater than 4 gm. a day, an amount comparable to that contained in a normal diet, may promote the excretion of sodium and chloride whereby significant losses of sodium and chloride occur and symptoms of crisis are precipitated. It may not be possible to prevent the occurrence of these effects of potassium with intakes of sodium and chloride as high as those provided by the

administration of 18 gm. of sodium chloride and 5 gm. of sodium citrate daily. If the intake of potassium is restricted to about 1.6 gm. a day, the requirement of sodium and chloride is materially diminished, and it becomes possible, although not necessarily desirable, to maintain patients with smaller doses of sodium salts than otherwise are needed and without injections of extract of adrenal cortex. Optimal therapeutic results demand not only the restriction of potassium but optimal rather than minimal doses of sodium salts and when possible, and certainly in emergencies, injections of an active extract of adrenal cortex. The arranging of a diet which will contain not more than 1.6 gm. of potassium requires careful planning. Such a diet is likely to be inadequate in calcium, phosphate, iron and vitamins B and G. To avoid complications from deficiencies of these factors, the diet should be supplemented suitably by the addition of calcium phosphate, some iron salt and a concentrate of vitamins B and G.—I. B.

Compensatory adrenal hypertrophy in the rat as influenced by sex, castration, time and thyroidectomy. Winter, C. A., and F. E. Emery, Anat. Rec. **66:** 401. 1936.

Castration causes adrenal hypertrophy in the male rat and atrophy in the female; the differences are statistically significant. When compensatory hypertrophy is produced in one adrenal by removal of the other, the percentage increase in size is the same in males and females, castrates and normals, so that the adrenals of the 4 groups retain essentially the same weight relationship one to another in the hypertrophied state as they occupied in the non-hypertrophied condition. When one adrenal is removed, hypertrophy is complete within 10 days, and in some cases apparently sooner, and is maintained at about the same level for the longest period observed in the present study (135 days). Thyroidectomy does not affect the degree of compensatory hypertrophy in the male rat. The factor (presumably from the anterior lobe of the pituitary) responsible for compensatory hypertrophy must act directly on the adrenal, or at least not through the intermediation of the thyroid. This adrenotropic factor is probably different from the one involved in adrenal hypertrophy produced by pituitary implants, for the latter is ineffective in thyroidectomized rats.—Authors' Abst., Wistar Bib. Service.

The effect of adrenalectomy on the cholesterol contents of tissues and organs. Yasue, S., J. Biochem. **24:** 483. 1936.

The amounts of cholesterol-esters in the brain and liver were lower in 4 cats 7 days after adrenalectomy than in 3 normal cats, while in kidney an increase was observed, and in lung and muscle no change. Total and free cholesterol were little affected. The change is not due to inanition alone, and can be prevented by administration of cortical extract prepared according to Swingle and Pfiffner.—D. L. T.

ENDOCRINE GENERAL

Studies on the opossum (Trichosurus vulpecula). Blood analyses and lipoid glandular constituents in normal and lactating opossums. Anderson, Doris, Australian J. Exper. Biol. & med. Sc. **15:** 17. 1937.

Chemical analyses were carried out on the citrated centrifuged blood of adult and half-grown male and female opossums and lactating opossums. The substances estimated included the non-protein nitrogen, sugar, total phosphorus, total lipoid, total cholesterol, total fatty acids, and lipoid phosphorus. Estimations were carried out both on the blood plasma and on the centrifuged corpuscles. In addition, the total lipoids, cholesterol, fatty acids and lipoid phosphorus were estimated in the liver, spleen, kidney and adrenal glands. No difference was found in the lipoid content of the liver or adrenals in males and females. In lactating opossums the blood sugar was found to be raised but there were no lipoid changes. The dry weight of the adrenal was found to be increased.—G. B.

Internal secretion as a factor in the origin of cancer. Loeb, L., E. L. Burns, V. Suntzeff and M. Moskop, Canad. M. A. J. **35:** 117. 1936.

Continued estrin treatment leads to appearance of mammary adenocarcinoma in mice, including males and non-breeding females. Lobules of gland tissue gradually develop, and the acini may secrete, but with continued stimulation tend rather to continue growing by mitosis and amitosis to form irregularly-coiled tubules. The generalized large-celled pre-cancerous tissue thus formed is the soil in which true carcinoma develops, often from multiple points of origin. The incidence is highest in strains in which breeding females are susceptible

to spontaneous mammary cancer, and is also correlated with the dose of estrin. A general discussion of the subject is built around these experiments.—D. L. T.

Hypoglycemic shock in the treatment of schizophrenia. Russell, L. W., Lancet **232:** 747. 1937.

Eight schizophrenics were subjected to a total of 416 periods of hypoglycemia. All showed improvement; 2 with remission, 3 much improved, and 3 showed little benefit from this treatment. In no instance were there untoward physical results.—L. B. S.

Changes in certain glands in chronic alcoholism (L'alteration de certaines glandes endocrines au cours de l'alcoholisme chronique). Villaret, M., L. Justin-Besançon, J. Delarue and H. P. Klotz, Presse méd. **44:** 2115. 1936.

A study on 60 cases of alcoholic polyneuritis. The thyroid seemed to pass through a stage of hyperactivity into hypoactivity. The females developed premature amenorrhea. The anatomic changes in the thyroids of 15 cases were quite variable. The ovaries showed more uniformly general sclerosis and absence of adult and primordial follicles as well as of corpora lutea; 50% were cystic. The changes in other glands were insignificant.—A. E. Meyer.

Estrogenic hormones in the treatment of vulvo-vaginitis in children. Berry, J. V. Canad. M. A. J. **36:** 396. 1937.

In a series of 25 cases treated with 1,000 I. U. of theelin tri-weekly, cures were obtained on the average in 45 days; recurrences occurred in 28%.—D. L. T.

GONADS

The preparation and purification of extracts containing the gonad-stimulating hormone of pregnant mare serums. Cartland, G. F., and J. W. Nelson, J. Biol. Chem. **119:** 59. 1937.

A gonadotropic hormone was obtained from pregnant mares serum by fractional precipitation with acetone or alcohol. The impurities were removed by iso-electric precipitation at pH 6.0 and the active material finally precipitated at a concentration of 70% alcohol or acetone. A dry white, water-soluble powder was obtained containing only 0.0025 mg. N per unit, which caused a fivefold increase in weight of ovaries of the immature rat in 96 hours.—J. M. L.

Estriolglycuronide. Cohen, S. L., G. F. Marrian and A. D. Odell, Biochem. J. **30:** 2250. 1936.

The water-soluble complex of estriol isolated from pregnancy urine has been isolated in crystalline form as a sodium salt. One of the alcoholic hydroxyls of the estriol is combined with the aldehyde group of glycuronic acid. The phenolic group of estriol is free, and the methyl ether of this group may be formed to yield estriol methyl ether on hydrolysis; moreover, Millon's test is positive. The reducing group of glycuronic acid is involved in the complex and liberated during hydrolysis. The method of preparation has been improved to yield up to 500 mg. from 30 litres of human pregnancy urine. The spectrographic properties are described. In adult ovariectomized mice the crystalline sodium salt had an activity of only 370 M. U. per mg., the corresponding value for pure free estriol being 10,700.—D. L. T.

Further observations on the estrogenic activity of synthetic polycyclic compounds. Cook, J. W., E. C. Dodds and W. Lawson, Proc. Roy. Soc. London, s.B., **121:** 133. 1936.

Eighteen new synthetic substances, derived from dibenzanthracene, chrysene, phenanthrene, benzanthracene, etc., have been tested for estrogenic activity, which is most commonly encountered in the dibenzanthracene series and is greatest in the dipropyldihydroxy derivative previously described.—D. L. T.

On normal and aberrant corpora lutea of the rhesus monkey. Corner, G. W., with the collaboration of G. W. Bartelmez and C. G. Hartman, Am. J. Anat. **59:** 433. 1936.

The corpus luteum of the rhesus monkey has during the first 3 weeks of pregnancy the same cytological structure as the corpus luteum of the non-pregnant cycle. It is composed of large lipid-containing granulosa lutein cells; the cells of the theca interna are not readily visible. The corpus of the non-pregnant animal begins to degenerate about the

time of onset of menstruation. The corpus luteum of pregnancy after the third week shows distinct alterations. The granulosa lutein cells lose their lipid inclusions and become smaller, the theca internal cells become visible in clumps in their original positions about the wall of the corpus luteum, and the pattern of folding of the follicular wall becomes more conspicuous. In non-pregnant animals aberrant corpora lutea, very similar in appeareance to the older corpora of pregnancy, are frequently found. They are not associated with the production of the so-called premenstrual changes of the endometrium. Another type of corpus aberrans is characterized by overgrowth of the theca interna cells and the persistence of wreaths or bars of granulosa lutein cells in the compact, lipid-free state.—Author's Abst., Wistar Bib. Service.

Capon comb growth-promoting substances ("male hormones") in human urine of males and females of varying ages. Dingemanse, Elisabeth, Helene Borchardt and E. Laqueur, Biochem. J. **31:** 500. 1937.

In human urine, androgenic substances are present chiefly in an inactive water-soluble form, which may be hydrolysed by autoclaving or by heating with acid, but not with alkali nor on standing at room temperature. The total quantity averages 40 to 60 I.U. (each equivalent to 0.1 mg. androsterone) per liter in both men and women during the active sexual period; post-menstrual increases were detected twice in 1 woman, but in 1 male subject there was no cyclicity. Smaller quantities are excreted by elderly men and women, and by boys (15 U per 1.) and girls (5 U) before puberty. In the urine of 5 women 6 to 8 months pregnant 8 to 30 U per 1. were found, and up to 12 U in urine of 3 women 1 year after ovariectomy. The fact that certain aged men excrete practically no androgenic substance makes it improbable that these principles are derived from the food. Human blood contains less than 20 U per 1. Animal urines are deficient in these substances, 1 U per 1. in the urine of pregnant mares, and similar or smaller amounts in dog urine or in the blood of steers and cows.—D. L. T.

Hormone therapy for ectopic testes. Farmer, A. W., Canad. M. A. J. **36:** 174. 1937.

A report of 32 cases treated with antuitrin-S, etc., in 16 of which descent of the testes occurred.—D. L. T.

The occurrence of an estrogenic substance in the sexual skin of monkeys. Fisher, R. B., P. L. Krohn and S. Zuckerman, Biochem. J. **30:** 2219. 1936.

Extracts of the sexual skin of 8 normal monkeys and 12 which had been injected with estrone were tested on ovariectomized mice; in 4 cases estrogenic activity was detected. In 1 untreated monkey this corresponded to 27 micrograms per kilo of tissue. In some instances estrogenic activity was found in the exudate of the sexual skin, or in the liver. The methods of extraction would not encompass combined forms of estrin such as glycuronides. The positive results are not due to estrin circulating in the blood, contained in the tissues examined. It is probable that estrin is selectively concentrated in the tissues upon which it acts. Active extracts were not obtained from sexual skin in the unswollen state.—D. L. T.

The effect of estrogenic hormones on lactation and on the phosphatase of the blood and milk of the lactating cow. Folley, S. J., Biochem. J. **30:** 2262. 1936.

Milk yield is lessened temporarily, but the percentage of fat and non-fatty solids is increased for some time. The serum calcium is transiently lowered, and serum phosphatase transiently but markedly increased.—D. L. T.

Precocious puberty following measles, encephalomyelitis and epidemic encephalitis, with a discussion of the relation of intracranial tumors and inflammatory processes to the syndrome of macrogenitosomia praecox. Ford, F. R., and Harriet Guild. Bull. Johns Hopkins Hosp. **60:** 192. 1937.

Three cases are described in which sexual precocity developed in children subsequent to encephalitis. The clinical symptoms resembled those observed in similar cases attributed to tumors of the pineal body.—A. G.

Variations in the ascorbic acid in the ovaries of cows, especially in the lutein tissue (Variations de l'acide ascorbique dans l'ovaire et spécialement dans le tissu lutéinique).

Giroud, A., I. Cesa, R. Ratsimananga and M. Rabinowicz, Compt. rend. Soc. de biol. **122:** 899. 1936.

The ascorbic acid content of corpora lutea of cows was estimated. It rose from 0.75 mg. per gm. of fresh tissue in the corpora of estrous to 1.03 at the stage of maximum activity and then declined. In the corpora of pregnancy, the maximum was 1.62 in the third month.—J. C. D.

Rhythmic diurnal variations in the estrous phenomena of the rat and their susceptibility to light and dark. Hemmingsen, A. M., and N. B. Krarup, Biologiske Meddelelser **13:** No. 7. 1937.

Using rats as experimental animals it was found that under ordinary conditions the mating reactions and muscular activity are maximally evident at night. When the rats were habituated to light during the night and darkness during the daylight hours there was a 12-hour shift in the rhythms. The vaginal changes were correspondingly shifted. The findings are related to the general principle of seasonal light-control of breeding activities.—R. G. H.

Significance of hormonal disturbances in the genesis of mastopathia cystica and epithelial metaplasias of the cervix (Die Bedeutung hormonaler Störungen in der Genese der Mastopathia cystica und der Epithelmetaplasien der Cervix.) Herold, L., and G. Effkemann, Zentralb. f. Gynäk. **61:** 1155. 1937.

Mature intact and castrate females and intact male rats were injected with 50 to 250 I.U. of estrogenic substance daily for 2 to 3 months. Two types of reaction were observed. All groups showed evidences of marked dilatation of ducts of the mamary glands, with epithelial proliferation in the ducts leading often to papillomatous growths and some metaplasia. Interstitial tissue showed hyperplastic changes. The changes are said to resemble human mammary fibrosis cystica. The second type of reaction occurred only in intact females, and in these cases there was evidence of extensive formation of corpora lutea. Here the cystic development extended to include the acinar tissue. The metaplastic appearances were not noted in this group. Apparently the action of both ovarian hormones produces a more normal sort of growth effect on the breast than that of the estrogenic hormone alone. The finding of cervical metaplasia is confirmed.—E. L. S.

The influence of a prolonged and increased follicle hormone-treatment on the vaginal mucosa of different animals (Wirkung einer langdauernden und gesteigerten Follikelhormon-Behandlung auf die Vaginalschleimhaut verschiedener Tiere.) Herold, L., and G. Effkemann, Zentralb. f. Gynäk. **61:** 1161. 1937.

The results of daily injection of 50 to 1000 international units of estrogenic hormone into mice and rats—adult intact, castrate, and immature—agree in showing increased thickness of the mucosa, increased amount of cornification of the surface layer, and extension into the submucosa of projections of the hyperplastic mucosa, but no metaplasia.—E. L. S.

The percutaneous activity of the sex hormones (Die perkutane Wirkung der Sexualhormone). Ito, M., S. Hajazu and T. Kon, Zentralb. f. Gynäk. **61:** 1094. 1937.

Using castrate and immature white rats, preparations of 'Enarmon' and 'Ovahormon' were applied in part as aqueous or oil solutions subcutaneously, and comparisons made with preparations rubbed into the ventral skin. The 60% alcohol solutions were best absorbed, followed in efficacy by solutions in water, cold cream, lanolin, and vaseline. End points are in terms of increased weight of secondaries and appearance of typical estrous smears.—E. L. S.

HYPOPHYSIS

Comparison of methods of extraction of the lactogenic hormone. Bergman, A. J., and C. W. Turner, J. Biol. Chem. **118:** 247. 1937.

The lactogenic hormone was extracted from desiccated anterior pituitary powder assayed by the McShaw Turner pigeon method. It was found that the 60 to 70% alkaline alcohol extraction method was superior to either the aqueous alkaline, and acetone or acetic acid methods.—J. M. L.

Excretion of gonadotropic substances in the urine during pregnancy. Browne, J. S. L., and E. M. Venning, Lancet **231:** 1507. 1936.

The excretion of gonadotropic substances were followed in 5 pregnant women. In 1

case, the specimens were collected daily, and in the others, 2 or 3 times weekly. The results of the normal curve of concentration differed from that generally accepted. There was a sharp rise in concentration between the 40th and 50th day after the beginning of the last menstrual period, the maximum concentration occurring between the 50th and 60th day. There was a rapid fall in concentration between the 60th and 85th day, the decline then became more gradual, a low level being reached on the 120th day. This was maintained throughout the rest of the pregnancy. The authors interpret this as being due to a transference of function from the ovary to the placenta. The concentration of prolan has been found to be low at this period (40 to 100 days); when the fetus (intra-uterine or extra-uterine) is dying, although the qualitative A. Z. test may be positive. The use of a quantitative determination of prolan, knowing the period of gestation, is suggested as a guide to therapy in cases of threatened abortion.—L. B. S.

Attempts to produce antigonadtropic substance by the use of serum or blood extract. Chen, G., Chinese J. Physiol. **11:** 329. 1937.

Two rabbits treated with human serum parenterally, 2 with extract of human blood, 7 with sheep serum and 2 with extract of sheep blood failed to develop anti-gonadotropic properties in their serum. Assays were conducted on immature rats, using 6 cc. of the serum to be tested and 2 mg. of anterior hypophyseal extract. Seventeen control rabbit sera showed some inhibition of sheep hypophyseal extract compared to the values obtained with saline in 3 assays.—S. Werner.

Results of further experiments with the anti-maturity hormone. Collip, J. B., Canad. M. A. J. **36:** 199. 1937.

Four young lambs were injected daily with a gonadotropic extract of sheep hypophyses for several months. At intervals, immature female rats were injected twice daily with 0.5 cc. of the sheep serum, and on the 4th, 5th and 6th days with a dose of the sheep gonadotropic extract normally capable of producing ovaries weighing 50 mg., with full estrus in 72 hours. The serum was given intraperitoneally and the hormone subcutaneously. The sera were at first synergistic, but after some 3 months treatment became strongly antagonistic to the hormone: they were also antagonistic to preparations from beef and (less markedly) hog hypophyses and from serum of pregnant mares, but not to preparations from human pregnancy urine. The antagonistic sera suppressed estrous cycles in normal female rats.—D. L. T.

The myometrial sensitizing action of follicular hormone (Ueber die wehenausloesende Wirkung des Follikelhormons). Druckrey, H., and H. Bachmann, Zentralb. f. Gynäk. **61:** 1091. 1937.

Cats and rabbits were subjected to laparotomy under pernocton; the gravid uterus (near term) was observed for ½ hour of quiet, tested to demonstrate only a single transitory response to ½ to 2 U of oxytocic factor from the posterior lobe, followed by 90 minutes of quiescence. Then after the intravenous injection of 200 M.U. of estrin the uterus showed immediate powerful contractions, still more marked after 400 U. Three of 7 animals had delivery of live litters within 80 to 100 minutes. The authors believe the estrogenic hormone serves only to release the labor mechanism to the stimulation of posterior lobe oxytocic factor. Clinical trials are under way.—E. L. S.

Potency of pituitary implants after several days in the muscles or peritoneal cavity. Emery, F. E., Anat. Rec. **66:** 253. 1936.

The data indicate that pituitary glands implanted in the muscles or peritoneal cavity of immature rats live for at least 8 days and still retain the gonadal-stimulating hormones. The histological structure of the anterior lobe was normal even 3 weeks after the implants were made. The possibility that such implants continue to secrete hormones is discussed.—Author's Abst., Wistar Bib. Service.

The alleged antidiuretic action of the pigmentary hormones of the pituitary gland. Fraser, A. M., J. Pharmacol. & Exper. Therap. **60:** 82. 1937.

Pituitary melanophore hormone, prepared by Stehle, and postlobin-V were assayed by the antidiuretic method of Burn. The results show that 0.001 pressor unit of postlobin-V has an activity more than 0.1 but less than 0.2 melanophore unit of the melanophore extract. Since the pressor action of 1 melanophore unit is 0.5% of 1 pressor unit of postlobin-V, it is evi-

dent that the antidiuretic action of the melanophore extract is due to contamination with pressor hormone. Alkali treatment of the melanophore hormone produced a total loss of pressor and antidiuretic activities.—E. L.

The effect of an anterior hypophyseal extract upon the serum calcium and phosphorus. Friedgood, H. B., and R. McLean, Am. J. Physiol. 118: 588. 1937.

The work confirms the previous work of Friedgood that injections of extract of the anterior hypophysis into normal guinea pigs raises the serum calcium. In 19 experimental animals the serum calcium was raised 0.9 mg.% above the average of the 15 control animals. No significant change was observed in the serum phosphorus.—Lena Lewis.

Simmonds' disease (anterior hypophysial insufficiency). Report of 2 cases with autopsy. Gallavan, Mae, and A. T. Steegmann, Arch. Int. Med. 59: 865. 1937.

Two case histories of Simmonds' disease, with autopsy findings, are reported. The first case showed the typical postmortem picture of fibrosis and almost complete atrophy of the anterior lobe of the hypophysis, fibrosis and marked atrophy of the adrenal glands, thyroid glands and gonads, as well as visceral microsplanchnia. The patient showed the characteristic history of onset following a complicated pregnancy and the loss of hair, teeth and sexual functions, and symptoms of weakness, chilliness and poor appetite, together with a subnormal temperature, a low basal metabolic rate, an increased sugar tolerance and anemia and a fatal termination in hypoglycemic coma. The lack of cachexia was explained as a result possibly of therapy. The central nervous system showed an uncharacteristic diffuse disease of the ganglion and glia cells and cellular defects in the cortex. The nucleus supra-opticus exhibited loss and degeneration of the ganglion cells. The second case presented several characteristics of Simmonds' disease—the onset of the menopause following toxemia of pregnancy, fatigue, mental disturbances, scanty body hair, a low basal metabolic rate and, at autopsy, an almost completely atrophic anterior lobe of the hypophysis and moderately atrophic adrenal and thyroid glands; but cachexia had never developed, death was not in hypoglycemic coma and there was no visceral microsplanchnia. The additional history of episodes of chills and fever and the finding of chronic and acute cystitis and pyelonephritis suggested that this intercurrent infection rather than Simmonds' disease was the terminating factor. In both cases pain in the knees was a prominent symptom.—I. B.

The meningeal relations of the hypophysis cerebri. The relations in adult mammals. Wislocki, G. B., Anat. Rec. 67: 273. 1937.

A method of studying the topography of the adult pituitary region is presented. This consists, after decalcification, in serially sectioning the base of the brain and skull with the pituitary completely in situ. The reasons are given for considering the observations derived from pituitaries studied by this method as more trustworthy than previous observations. The conclusion is reached that in a variety of mammals (rat, rabbit, cat, dog, monkey, and man) the subarachnoid space encircles the infundibular stalk, but does not surround the body of the hypophysis. When a sella turcica exists, the subarachnoid space penetrates into it for only a short distance (dog and cat, anteriorly) or not at all (rabbit, monkey, and man). The capsule of the body of the pituitary gland, the dura lining the sella, and the periosteum covering the bone have fused in all of the mammals investigated. Consequently the subdural space does not extend around the body of the gland, but terminates, similarly to the subarachnoid space, as a collar surrounding the hypophyseal stalk. Shrinkage during dehydration of specimens often causes clefts, which can be shown to be artefacts, to arise in the fused layers enclosing the body of the gland.—Author's Abst., Wistar Bib. Service.

Comparative action of injections of estrin and a combination of estrin and anterior pituitary-like substance on the anterior hypophysis. Wolfe, J. M., Anat. Rec. 68: 237. 1937.

In mature and immature male and female rats with intact gonads, the simultaneous injection of large amounts of APL (anterior pituitary-like substance) along with estrin resulted in a histologic response in the pituitary which was modified from that obtained by injecting the same amounts of estrin alone. The degree of the weight increase of the pituitary and the degree of degranulation of the chromophilic cells, together with a reduction in their relative percentages and an increase in that of the chromophobes, were more marked in the animals which received estrin alone than in those which received both estrin and APL. In castrated

female rats the comparative histologic changes obtained in the anterior hypophysis by the injection of estrin alone and by combinations of estrin and APL were identical. Injection of estrin into castrated and normal female rats elicited similar weight increases and histologic changes in the anterior hypophysis. Injection of progesterone in castrated female rats, simultaneously with estrin, did not result in any pituitary response different from that obtained by the injection of estrin. Cell counts were made on all pituitaries studied; the results were analyzed statistically.—Author's Abst., Wistar Bib. Service.

Tumour growth in hypophysial dwarfism. Zondek, B., Lancet **232:** 689. 1937.

Using prolonged treatment with follicular hormone to impair the function of the growth hormone of the anterior pituitary of the rat, malignant tumors were then implanted into these animals in an attempt to impair the rate of growth of these tumors. It was possible, through these experiments, to illustrate the fact that the growth hormone of the anterior pituitary has no important effect upon the growth of malignant tumors.—L. B. S.

PANCREAS

Cerebral lesions in hypoglycemia. Baker, A. B., and N. H. Lufkin, Arch. Path. **23:** 190. 1937.

In conjunction with the study of human material presenting hypoglycemic symptoms, 6 rabbits were selected and hypoglycemic convulsions were induced by the injection of from 8 to 70 U of insulin. Convulsions were allowed to continue for varying lengths of time up to 1 hour and then terminated by intravenous injections of 10 cc. of a 50% dextrose solution. Hypoglycemic attacks were produced once to twice each week for from 6 to 8 weeks. By comparison with the normal histologic picture, very little change could be found in the brains of these hypoglycemic animals. In the human cases studied post-mortem, there appeared extensive and widespread alteration of the ganglion cells, both the cytoplasm and nucleus being involved. The character of the change suggests post-mortem alteration. A final feature of study was the presence of tiny hemorrhages scattered irregularly throughout the brain. These seemed most numerous in patients who had the most severe convulsive seizures.—L. B. S.

The effect of insulin on carbohydrate formation in the liver. Bach, S. J., and E. G. Holmes, Biochem. J. **31:** 89. 1937.

The experiments were conducted with the Dixon-Keilin differential manometer on surviving slices of livers of fasted rats in Ringer's solution buffered with bicarbonate. The amount of fermentable reducing substances in the tissue increased from 5.2 mg. per gm. fresh liver to 7.5 mg. in 1 to 5 hours (average of 19 experiments, with very wide variations). This increase was partly inhibited by addition of insulin, 0.2 to 3.3. U per cc. of medium. The average inhibition was 40%, and was not correlated with insulin concentration. The insulin did not increase the oxygen consumption nor the respiratory quotient (0.6). The apparent carbohydrate synthesis was increased in the presence of added lactate, pyruvate, alanine, aspartic acid, or arginine; glycine, however, was inhibitory. Lactate, pyruvate, alanine and glycine increased the oxygen consumption. Insulin inhibited the formation of carbohydrate from alanine, but not from lactate or pyruvate; it also inhibited the formation of urea, either from alanine or in the absence of added substrates. In the presence of added alanine, insulin lowered the R.Q. It is concluded that insulin inhibits the new-formation of carbohydrate from alanine, etc., by deamination.—D. L. T.

Crystalline insulin. Barach, J. H., Ann. Int. Med. **10:** 1335. 1937.

The glycolytic action of crystalline insulin prepared according to the method of Sayhun was studied in 21 cases of diabetes and compared with the action of standard insulin and with protamine insulin in 1 case. No disadvantages were found either in administration or effects. Its sugar-reducing value as shown by hourly determinations of blood and urine sugar was greater in 12 and less in 9 of the 21 cases. The effect was more prolonged in middle-aged and older diabetics than in younger ones. Reduction of blood sugar to the 50 mg. zone was obtained without the manifestation of reactions characteristic of standard insulin. Insulin reactions or insulin shock occurred only one-fourth as often as when standard insulin was used. Because of its slower action and lessened tendency to produce shock, larger and fewer doses may be used.—R. R. Durant.

Unusual glycogen storage in a case of diabetes mellitus. Brian, E. W., A. J. Schechter and E. L. Persons, Arch. Int. Med. **59:** 685. 1937.

The case reported occurred in a diabetic white man of 24 who was admitted to the hospital in severe diabetic acidosis. The patient died within 12 hours after admission. Post-mortem examination revealed hepatomegaly associated with a large amount of glycogen. Two possible explanations for this unusual condition are considered: first, this was a case of glycogen storage disease (von Gierke) in a subject who had survived to young adulthood, when diabetes developed, and, second, the glycogen was stored in the liver under the influence of large doses of insulin plus a high intake of carbohydrate.—I. B.

The trend of diabetes in Saskatchewan, 1905 to 1934. Chase, L. A., Canad. M. A. J. **36:** 366. 1937.

The death-rate from diabetes in Saskatchewan is low, the population being largely rural and of low average age, but is increasing, notably in females over 50. Pure-blooded Indians are not subject to diabetes.—D. L. T.

The mechanism of convulsions in insulin hypoglycemia. Drabkin, D. L., and I. S. Ravdin, Am. J. Physiol. **118:** 174. 1937.

Forty experiments were performed on 31 dogs weighing 6 to 26 kg. In each case a study was made of the response, during a 4-hr. period, following the intravenous injection of 20 U per kg. of insulin. The previous treatment of the animals varied but fell into 5 main groups: 10 experiments on 6 hydrated animals (water ad libitum plus 40 to 80 cc. water per kg. per day by stomach tube for 5 to 7 days); 9 experiments in 6 dehydrated dogs (deprived of water but not of food for 5 to 7 days); 13 experiments on 11 hydrated dogs which had previously undergone bilateral removal of the stellate ganglia; 5 experiments on 5 hydrated dogs which had previously undergone sectioning of both splanchnics at the level of the 10th rib; 3 experiments on 3 hydrated dogs which had been subjected to both bilateral stellate ganglionectomy and bilateral section of the splanchnics. Total reducing substances and hemoglobin were determined. Cerebrospinal pressure measurements were made by means of a water manometer with the animal under light ether or amytal anesthesia. The previous state of 'hydration' of an animal has a profound effect upon the incidence of convulsions in insulin hypoglycemia. 'Dehydrated' animals do not have convulsions. The experiments disclosed the following sequence of events leading to convulsions in hyperinsulinism: severe hypoglycemia → anhydremia → rise in cerebrospinal fluid pressure to a critical level → convulsions. In all probability, other factors also function in the cycle, although they have not been disclosed. The administration of such agents as sodium arabinate, the practice of dehydration and excision of the stellate ganglia interfered with the above cycle at different points, and thereby presented a convulsive response in insulin hypoglycemia. The excision of both stellate ganglia modified the animal's response to insulin by delaying and markedly reducing the resultant hypoglycemia. The further operation of bilateral splanchnic section once again rendered the animal normally reactive to insulin probably by reducing the secretion of epinephrin in response to hypoglycemia.—K. Brownell.

The antiketogenic action of glucose in the absence of insulin. Mirsky, I. A., J. D. Heiman and R. H. Broh-Kahn, Am. J. Physiol. **118:** 290. 1937.

Studies were made of 28 dogs on which pancreatectomy had been performed 96 hr. before the actual experiment. The animals were divided into 2 groups: In one the kidneys were removed under sodium amytal anesthesia immediately before the experiment; the 2nd group was phlorhizinized 24 hr. before the actual experiment. A 33% solution of glucose (1 gm. per kg.) was injected into the femoral vein every half hour. Blood samples were collected from the femoral artery at 2 hr. and immediately before the injection and at 2-hour intervals thereafter. Blood was examined for 'total acetone', blood sugar and total solids. Where the kidneys remained intact, urine samples were collected at the same intervals and ketones determined. For control purposes an isosmotic NaCl solution was injected. The intravenous injection of such large amounts of glucose to anesthetized nephrectomized-depancreatized dogs, to unanesthetized, depancreatized dogs and to phlorhizinized-depancreatized dogs, in all of which the presence of insulin was entirely excluded, resulted in a disappearance of ketone bodies. This antiketogenic action of glucose is attributed to deposition of glycogen in the liver and a consequent cessation of ketone formation. The administration of phlorhizin to the fasted depancreatized dog resulted in a rapid drop in the blood sugar and a concomitant aggravation of the ketonemia. An inverse relationship exists between the sugar and ketone bodies of the

blood. The degree of ketonuria is no index of the ketone body content of the blood although a change in the latter produces a similar change in the former. These studies suggest that the metabolic disturbance in pancreatic diabetes is due to an alteration in the balance between hepatic glycogenesis and glycogenolysis. The associated production of ketone bodies is attributed to a compensatory acceleration in the catabolism of fatty acids and proteins in the liver in consequence of a decrease in the amount of carbohydrate available for oxidation by the liver per se.—K. Brownell.

Studies on the physiology of lactation. The induction of lactation in depancreatized dogs. Nelson, W. O., H. E. Himwich and J. F. Fazikas, Anat. Rec. **66:** 201. 1936.

Nine completely depancreatized dogs that were maintained on insulin and an adequate diet received injections of lactogenic hormone. Milk secretion occurred in 5 of these animals on the fourth to sixth days of treatment. Four animals failed to lactate although injections were continued for 11 to 15 days. In the absence of detailed information concerning the reproductive histories of our animals their cyclic condition was estimated by a study of ovaries, uteri and mammary glands removed prior to the initiation of treatment. Of the animals that lactated 4 were pseudopregnant and 1 had been pregnant. In these bitches the ovaries contained fairly recent corpora lutea, the uterine endometria showed proliferation, and the mammary glands were well developed. In the instances where no milk was secreted the animals were judged to be in the anoestrum. Their ovaries contained very old corpora lutea, the uterine endometria were very low, and the mammary glands were involuted. These experiments show that the depancreatized bitch which is maintained in good condition by adequate diet and insulin will respond to the lactogenic hormone in the same fashion as the normal bitch. The deciding factor in the response of both the normal and the diabetic animal to the lactogenic hormone has been an adequate proliferation of the mammary glands.—Authors' Abst., Wistar Bib. Service.

Further observations on the use of protamine zinc insulin in diabetes mellitus. Rabinowitch, I. M., A. F. Fowler and A. C. Corcoran, Canad. M. A. J. **36:** 111. 1937.

Protamine zinc insulin has remarkably little tendency to cause reactions even when the blood sugar falls below the hypoglycemic level. The preparation is stable; precautions to insure its being injected at the proper pH are described. Rapid decrease in blood sugar is obtainable with large doses, so that it may be used even in emergencies. Diet affects its efficiency markedly: there is evidence that carbohydrate tolerance may be significantly improved. The action is so prolonged that a moderately severe diabetic may be controlled by large single doses at intervals of 24 hours or more; in 121 of 132 cases, daily injections were sufficient.—D. L. T.

Influence of insulin on the sugar level of lymph glands in the silkworm (Bombyx mori). Wenig, K., and J. Joachim, Biochem. Ztschr. **285:** 98. 1936.

In accordance with Hemmingsen the total reduction (in glucose equivalents) and fermentable sugar of silkworm lymph was found to be 106 ± 5.3 mg.% and 22.5 ± 5.5 mg.%, respectively. Within 1 hour after injection of 0.1 clinical units of insulin the fermentable sugar disappeared from the lymph. The total reduction remained unchanged. Therefore it is concluded that insulin does not influence the sugar in lymph.—F. Breuer.

Studies on the constitution of insulin. II. Further experiments on reduced insulin preparations. White, A., and K. G. Stern, J. Biol. Chem. **119:** 215. 1937.

Purified insulin was treated for 150 minutes at 30° and a pH 1.85 with thio-glycolic acid. There were no significant differences found between the reduced and the original insulin as regards total sulphur, amino nitrogen, tyrosine, iso-electric point or molecular weight. Reoxidation of the reduced product results in the disappearance of the free sulphydryl groups, and almost complete loss of physiological activity. Treatment of the original insulin under comparable conditions leads to a loss of from 20 to 38% of the physiological activity.—J. M. L.

Coincident diabetes and tuberculosis. Wilson, Ruth W., Pennsylvania M. J. **40:** 342. 1937.

The author presents 10 cases of tuberculosis and diabetes representing all stages of tuberculosis. These were followed from 1 to 8 years. In 9 of these cases the course of the disease was identical with that of a similar stage of involvement and activity of the tuberculosis without complicating diabetes. Two acute ulcerative cases contained in the series healed well when pneumothorax was established; the acute fibrotic case healed perfectly without surgery; the

chronic fibrocaseose case with cavitation became quiescent but continued to drain positive sputum from uncontrolled cavities; the minimal and pleuritis cases became promptly quiescent, and these patients have remained well; the hopelessly advanced moribund patients died in the usual expected length of time. As death approached, 1 patient developd a progressively higher carbohydrate tolerance and needed no insulin. The patient with acute ulcerative tuberculosis who could not take pneumothorax and left the hospital against advice before other collapse therapy could be attempted, died sooner than patients with the same type of tuberculous activity when uncomplicated although this type (so-called galloping consumption) is usually fatal even when unaccompanied by diabetes. In this case the diabetes probably only added speed to the inevitable decline. No unusual skill is needed in the treatment of these associated diseases, but the 2 conditions must be treated in the same institution and allowances made in insulin dosage for bed rest, moderately generous diet, and the toxemia that often occurs from autotuberculinization during the early compression period of collapse therapy. Carbohydrate tolerance varies so greatly with each case that no one can anticipate what the insulin requirements for any type will be even under what appear to be identical conditions.—I. B.

PARATHYROID

Further experience in the diagnosis of hyperparathyroidism, including a discussion of cases with a minimal degree of hyperparathyroidism. Albright, F., H. W. Sulkowitch and Esther Bloomberg, Am. J. M. Sc. 6: 800. 1937.

This paper is based on 35 cases of hyperparathyroidism proved by operation and studied by the authors. All but 2 of the 22 cases first diagnosed by the authors had clinical findings entirely different from cases being reported in the literature from other clinics. The conclusion is made that the series is so large because cases are being recognized which are being missed elsewhere. There are 2 groups of cases in particular which apparently frequently escape diagnosis. These are cases with no demonstrable bone disease and cases of moderate severity. The presence or absence of bone disease is not a function of the degree of hyperparathyroidism, some of the severe cases not having it, some of the mild ones having it. Patients with bone disease and high serum phosphatase levels as a rule develop postoperative hypocalcemia; other cases seldom do. The term 'border-line' has been applied to mild cases of hyperparathyroidism and refers to the degree of hyperparathyroidism, not to the symptoms. In the diagnosis of such cases the following points may be important: the serum protein determination, so that allowance can be made for the 'bound' calcium in interpreting the total calcium value; a persistently low serum phosphorus level; the calcium excretion in the urine; the composition of the stone; repeated blood determinations.—R. G. H.

The influence of parathyroid hormone, urea, sodium chloride, fat and of intestinal activity upon calcium balance. Aub, J. C., Dorothy M. Tibbetts and Regina McLean, J. Nutrition 13: 635. 1937.

Metabolic observations on human subjects justify the following conclusions: Parathyroid oversecretion has previously been shown to have no influence on fecal calcium excretion. These observations indicate that it has no consistent effect on intestinal absorption of calcium. An excellent organic solvent like urea does not influence calcium absorption from the intestine. Urea ingestion, however, does elevate the blood calcium level somewhat in exophthalmic goiter and hyperparathyroidism, and increases urinary calcium excretion in all cases. This increased excretion is independent of diuresis. Calcium excretion in normal subjects is independent of voluntary constipation, cascara catharsis, or the ingestion of agar, large amounts of fat, or of sodium chloride. Calcium excretion is, therefore, independent of many factors which might be expected to affect it, and in health remains at a remarkably constant level considering the vast available store in the bones. That some factor greatly increases calcium absorption from the intestines is again made obvious in these observations, but its nature is still unknown.—Authors' Abst., Wistar Bib. Service.

Studies in mineral metabolism. II. Calcium and the kidney, experimental. Chown, B., Margaret Lee and J. Teal, Canad. M. A. J. 35: 513. 1936.

Intraperitoneal injection of 2 U of parathormone daily into 5 day-old rats leads within 24 hours to appearance of fine calcium particles within the collecting tubules, and somewhat later to formation of small, dense crystalline calcium deposits lying interstitially close to the tubules, probably in lymphatics; these masses may eventually be extruded into the tubules.—D. L. T.

An apparent instance of parathormone inactivity. Mathewson, F. A. L., and A. T. Cameron, Canad. M. A. J. **36:** 141. 1937.

In a case of purpura, 300 U of parathormone was given within 75 hours without changing serum calcium or phosphate from their normal levels; the potency of the preparation was established.—D. L. T.

The influence of parathyroid extract on the blood sugar (Sur l'action hypoglycémiante de l'extrait parathyroidien). Olmer, J., and J. E. Paillas, Presse méd. **44:** 1418. 1936.

Parathyroid extract lowers the blood sugar in diabetic patients. Experiments and histologic studies in rats suggest that the extract stimulates the islands of Langerhans.—A. E. Meyer.

THYROID

Histological variations in fetal calves' thyroids and a comparison with maternal thyroids. Abbott, A. C., and J. Prendergast, Canad. M. A. J. **36:** 228. 1937.

Colloid, vesicle formation and vacuolation were found as early as the second month of intra-uterine life (much earlier than in the human fetus); hyperplasia was found in the fourth month, and differentiation was complete by the end of the sixth.—D. L. T.

Cytological changes in the rat thyroid following exposure to heat and cold, and their relationship to the physiology of secretion. Baillif, R. N., Am. J. Anat. **61:** 1. 1937.

Albino rats of a single genetic strain were divided into 3 groups; one of these was exposed to cold, the second to heat, and the third preserved as control. Thyroids of these groups were then compared cytologically. Cold exposure caused an increase in height of follicular cells. Filamentous mitochondria increased in number, and the Golgi net fragments. The chromatin material in these cells became concentrated into sharply staining blocks. Lethal exposure produced a marked loss of mitochondria. Portions of the follicular walls were destroyed, liberating colloid into the interfollicular spaces. Heat exposed thyroids showed an increase in stored colloid, and flattening of follicular cells. Mitochondria were decreased in number, and became granular in form. The Golgi material appeared as a closely meshed net adjacent to the nuclear membrane. Lethal heat exposure caused cytoplasmic collapse, with complete disappearance of both mitochondria and Golgi material. Secretion production was initiated by juxtaposition of several mitochondria and a portion of the Golgi net. These dissolved to form a secretion granule. The granule then transformed into a secretion vacuole which was discharged into the follicle. Colloid resorption was normally intra-cellular. Islets of non-follicular epithelium are described which transform directly into new follicles on cold exposure, but remain practically unchanged on exposure to heat.—Author's Abst., Wistar Bib. Service.

The histophysiological reactions of the thyroid in hypertensive states (Les réactions histophysiologiques de la thyroïde au cours des états hypertensifs). Bastenie, P., Compt. rend. Soc. de biol. **122:** 815. 1936.

Thyroids were examined from 113 cases of hypertension, 84 of which were the essential type and 29 the renal. In the latter, the histological picture was that of a gland in reduced activity with accumulations of colloid. In essential hypertension, there were signs of increased activity as though the gland was stimulated by the same factors that caused the hypertension.—J. C. D.

The early development of the thyroid gland in the dog with especial reference to the origin and position of accessory thyroid tissue within the thoracic cavity. Godwin, M. C., Anat. Rec. **66:** 233. 1936.

The early thyroid anlage of the dog is peculiar for its precocious formation of branching cell cords from the thyroid plate. The potential lumen in the cellular cords and the cavities later appearing in the thyroid follicles represent the surface of the thyroid plate. The pharyngeal surface of the thyroid plate decreases by its downward growth and by the inward expansion of surrounding material until it is attached to the pharyngeal floor by a narrow stalk. The stalk may be patent and has been found to give rise to certain cysts within or without the thyroid lobes. Some of the caudal tips of the early much branched thyroid anlage are in intimate association with the aortic sac. Such closely associated pieces remain with the aortic sac during its relative descent and form thyroid tissue upon the aortic arch of the adult. The thyroid cords are for a time during development cupped about the aortic sac and caudal ends of the common carotid arteries. Accessory thyroid tissue is found at variable levels in the anterior mediastinum

as a result of caudal displacement of thyroid tissue by the descent of the aortic sac and adjoining vessel.—Author's Abst., Wistar Bib. Service.

Relation between the thyroid gland and the development of salamander larvae under the influence of different temperatures (Über die Beziehungen zwischen Schilddrüse und Entwicklung bei Salamanderlarven unter dem Einfluss verschiedener Temperaturen). Hartwig, H., Arch. f. Entwcklngsmechn. d. Organ. **134:** 562. 1936.

The effects of various temperatures upon the thyroid gland and upon the development of fire salamander (Salamandra maculosa) larvae were studied. The temperature was held constant within 5° at six intervals from 0 to 30°C. The optimum temperature for both thyroid development and function and for rate of development of the larvae is between 15 and 20°C. Microscopic examination showed that extreme cold results in a small thyroid with little colloid. With extreme heat the thyroid is also small but contains relatively more colloid. The development of the epidermis parallels that of the thyroid. In extreme cold (0-5°) metamorphosis cannot be brought about by thyroxin.—R. F. Blount.

The thyrotoxic heart with special reference to masked hyperthyroidism. Hay, J., Lancet **231:** 1377. 1936.

The author believes we are dealing with a toxemia which persists from the 4th to 7th decade of life. Based on a critical analysis of a large mass of clinical material, certain features are recognized as characteristic of thyrotoxicosis: increase in B.M.R.; systolic B.P. tends to rise and the rise in diastolic pressure is either negligible or slight, the result being an increase in pulse pressure; in addition to tachycardia there is a liability to arrythmias, more particularly that of auricular fibrillation. Masked hyperthyroidism is a term which signifies that the usual signs and symptoms of hyperthyroidism are obscured by the more obvious indications of some other clinical entity. Recognition of a low-grade thyrotoxicosis depends on being alert to indications. Some of them are: cardiac deviations from normality—in fact, auricular fibrillation or flutter in patients with normal B.M.R. under 50, may be the earliest sign of thyrotoxicosis; though no exophthalmos, there is a peculiar staring quality or brightness, such patients being restless, eager, alert, and difficult with a leaning to emotional instability; transient glycosuria; G.I. complaints such as nausea, vomiting, loss of appetite, abdominal pain, diarrhea, difficulty in swallowing, loss of weight even to emaciation.—L. B. S.

The management of severe hyperthyroidism. Lahey, F. H., Surg., Gynec. & Obst. **64:** 304. 1937.

The author has performed 15,200 thyroid operations on 13,000 patients with the low mortality rate of 0.85%. Of those succumbing, 40% died in thyroid crisis, 10% were thyrocardiacs dying of cardiac failure, and 10% died of emboli. The remainder died of various causes not related to this paper. In view of this extensive experience, the author believes that if the patients were not permitted to proceed to a thyroid crisis, a severe stage reached in hyperthyroidism, or hyperthyroidism permitted to exist too long in patients with grossly crippled hearts, most of the mortality would be avoided.—L. B. S.

Failure of thyroidectomy to influence the follicular components of the immature rat ovary. Leonard, Olive L., and S. L. Leonard, Anat. Rec. **68:** 24. 1937.

Immature female rats deprived of their thyroid glands for 6 to 8 days, showed no significant change in the total number of ovarian follicles when compared with the controls. Neither was there a significant change in the number of vesicular follicles following the operation. While it is known that thyroidectomy enhances the effectiveness of injected follicle-stimulating hormone, the removal of the thyroids does not enhance the follicle-stimulating hormone produced by the animal's own hypophysis.—Authors' Abst., Wistar Bib. Service.

Studies of the action of thyroid hormone on cells in tissue culture (Expériences sur l'action de l'hormone thyroïdienne vis-a-vis des cellules cultivées hors de l'organisme). Verne, J., and D. Odiette, Compt. rend. Soc. de biol. **122:** 988. 1936.

Fibroblasts and nervous tissues were grown in a medium containing embryonic tissue fluids and chicken plasma. The addition of thyroxin to this medium did not influence the rate of growth. If the normal plasma was replaced by plasma from chickens which were receiving thyroxin injections there was a marked stimulation of cell division and growth. This was true through the first week of the injections but the plasma began to lose its stimulating property after that time regardless of whether the donor continued to receive thyroid or not.—J. C. D.